PHARMACOGNOSTICAL, PHYTOCHEMICAL STUDIES INCLUDING ISOLATION OF LUTEIN AND ITS SUN PROTECTION FACTOR, IN VITRO ANTI-INFLAMMATORY, ANTI-ARTHRITIC AND ANTI-OXIDANT ACTIVITY OF

Commelina benghalensis Linn.

A Dissertation submitted to

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MASTER OF PHARMACY

IN

BRANCH – III PHARMACOGNOSY

Submitted by
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MAY 2018

CERTIFICATE

This is to certify that the dissertation entitiled **"PHARMACOGNOSTICAL, PHYTOCHEMICAL STUDIES INCLUDING ISOLATION OF LUTEIN AND ITS SUN PROTECTION FACTOR, INVITRO ANTI-INFLAMMATORY, ANTI-ARTHRITIC, AND ANTI-OXIDANT ACTIVITY OF *Commelina benghalensis L.*"** is a bonafide work done by **Mr.A.IYAPPAN (261620703), DEPARTMENT OF PHARMACOGNOSY, COLLEGE OF PHARMACY, MADURAI MEDICAL COLLEGE, MADURAI-625020** in partial fulfilment of the The Tamilnadu Dr.M.G.R Medical university rules and regulation for award of **MASTER OF PHARMACY IN PHARMACOGNOSY** under my guidance and supervision during the academic year 2017-2018.

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CERTIFICATE

This is to certify that the specimen brought by Mr.A.Iyappan, II M.Pharm, Department of Pharmacognosy, College of Pharmacy, Madurai Medical College, Madurai is identified as *Commelina benghalensis Linn.* belonging to the family Commelinaceae.

Station : Madurai.

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Date : 11.08.2017.

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NAME : Commelina benghalensis

FAMILY : Commelinaceae

GENUS : Commelina

SPECIES : benghalensis

LOCALITY : Madurai Medical College, Madurai

DATE : 11.08.2017

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CONTENTS

CHAPTER-1

INTRODUCTION

INTRODUCTION

Plants have been used for medicinal purposes long before recorded history. Ancient Chinese and Egyptian papyrus writings describe medicinal uses for plants as early as 3000 BC. Indigenous cultures such as African and Native American used herbs in their healing rituals, while other developed traditional medical systems such as Ayurveda and traditional Chinese medicine in which herbal therapies were used.

The word **"herb"** has been derived from the Latin word, **"herba"** and an old French word **"herbe"**.

Recently there has been a shift in universal trend from synthetic to herbal medicine, which we can say 'Return to Nature'. Medicinal plants have been known for millennia and are highly esteemed all over the world as a rich source of therapeutic agents for the prevention of diseases and ailments. Nature has bestowed our country with an enormous wealth of medicinal plants; therefore India has often been referred to as the Medicinal Garden of the world. Countries with ancient civilizations such as China, India, South America, Egypt, etc. are still using several plant remedies for various conditions. In this regard India has a unique position in the world, where a number of recognized indigenous system of medicine viz., Ayurveda, Siddha, Unani, Homeopathy, Yoga and Naturopathy are being utilized for the health care of people. No doubts that the herbal drugs are popular among rural and urban community of India. The one reason for the popularity and acceptability is belief that all natural products are safe. The demand for plant based medicines, health products, pharmaceuticals, food supplement, cosmetics etc are increasing in both developing and developed countries, due to the growing recognition that the natural products are non-toxic, have less side effects and easily available at affordable prices.

Now a days, there is a revival of interest with herbal-based medicine due to the increasing realization of the health hazards associated with the indiscriminate use of modern medicine and the herbal drug industries is now very fast growing sector in the international market. But unfortunately, India has not done well in this international trade of herbal industry due to lack of scientific input in herbal drugs. So, it would be appropriate to highlight the market potential of herbal products and that would open floodgate for development of market potential in India.

Historically herbal drugs were used as tinctures, poultices, powders and teas followed by formulations and lastly as pure compounds. Medicinal plants or their extracts have been used by humans since time immemorial for different ailment and have provided valuable drugs such as analgesics (morphine), antitussives (codeine), antihypertensives (reserpine), cardiotonics (digoxin), antineoplastics (vinblastine and taxol) and antimalarials (quinine and artemisinin). Some of the plants which continue to be used from mesopotamian civilization to this day are *Cedrus* spp., *Cupressus sempervirens, Glycirrhiza glabra, Commiphora wightii* and *Papaver somniferum.* About two dozen new drugs derived from natural sources were approved by the FDA and introduced to the market during the period 2000-2005 and includes drugs for cancer, neurological, cardiovascular, metabolic and immunological diseases and genetic disorders. Seven plant derived drugs currently used clinically for various types of cancers are taxol from *Taxus* species, vinblastine and vincristine from *Catharanthus roseus*, topotecan and irinotecan from *Camptotheca accuminata* and etoposide and teniposide from *Podophyllum peltatum*. It is estimated that the worldwide market potential for herbal drugs is around US$40 billion. Mostly herbal drugs are collected from the wild and relatively few species are cultivated. Overexploitation of plants, particularly when roots, tubers and bark are used for commercial purposes, has endangered 4,000 to 10,000 species of medicinal plants. To counter overexploitation of natural resources and the consequent threats to biodiversity, alternative

biotechnological methods and sustainable practices have been recommended. Several world organizations and governments have established guidelines for the collection and utilization of medicinal plants.

TRADITIONAL USE OF MEDICINAL PLANTS

Traditional medicine is the sum total of the knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures used in the maintenance of the health, prevention of diseases and improvement of physical and mental illness. In practice, traditional medicine refers to the acupuncture (China), ayurveda (India), unani (Arabic countries), traditional birth attendant's medicine, mental healer's medicine, herbal medicine, and various forms of indigenous medicine.

Knowledge of the medicinal plants used in the drugs of traditional systems of medicine has been of great significance, especially as a lead for the discovery of new single- molecule medicines for modern system of medicine. To determine the chemical nature of such compounds, isolation of a substance in pure form using various separation techniques, chemical properties and spectral characteristics are a prerequisite for establishing its correct structure. Thus medicinal plants are used in crude or purified form in the preparation of drugs in different systems. Structural novelty and new modes of action are common features of plant drugs. This has been shown by anticancer agents like vinblastine, vincristine, and paclitaxel, cardiovascular agent like forskolin, anti-HIV agents like calanoid, and antihyperlipidemic agents like guggulsterones.

WHY HERBAL MEDICINE?

Herbal medicines are being used by about 80% of the world population primarily in the developing countries for primary health care. They have stood the test of time for their safety, efficacy, cultural acceptability and lesser side effects. The chemical constituents present in them

are a part of the physiological functions of living flora and hence they are believed to have better compatibility with the human body. Ancient literature also mentions herbal medicines for age-related diseases namely memory loss, osteoporosis, diabetic wounds, immune and liver disorders, etc. for which no modern medicine or only palliative therapy is available. These drugs are made from renewable resources of raw materials by eco friendly processes and will bring economic prosperity to the masses growing these raw materials.

ROLE OF WHO IN HERBAL MEDICINE

Two decades ago, WHO referred to traditional health systems (including herbal medicine) as 'holistic' – 'that of viewing man in his totality within a wide ecological spectrum, and of emphasizing the view that ill health or disease is brought about by an imbalance or disequilibrium of man in his total ecological system and not only by the causative agent and pathologenic evolution, probably implying that the indigenous system drugs (including herbal medicine) restore the imbalance or disequilibrium leading to the cure of ill health or disease. Such an attitude sent signals that WHO as an organization has failed to provide leadership to establish traditional systems of medicine which provide health care to about 80% of the world population. However, it helped the inclusion of proven traditional remedies in national drug policies and regulatory approvals by developing countries. The World Health Assembly continued the debate and adopted a resolution (WHA 42.43) in 1989 that herbal medicine is of great importance to the health of individuals and communities. Consequently, in 1991 WHO developed guidelines for the assessment of herbal medicine, and the same were ratified by the 6th International Conference of Drug Regulatory Authorities held at Ottawa in the same year. The salient features of WHO guidelines are: (i) Quality assessment: Crude plant material; Plant preparation; Finished product. (ii) Stability: Shelf life. (iii) Safety assessment: Documentation of

safety based on experience or/and; Toxicology studies. (iv) Assessment of efficacy: Documented evidence of traditional use or/and; Activity determination (animals, human).

EXPLORATION OF MEDICINAL PLANTS

Plants are a great source of therapeutic molecule. In the early 20th century, taxonomic surveys established the identity of plants, followed by ethnomedical surveys documenting the use of plants as medicine and other uses. The identification of active principles of medicinal plants leads to the use, misuse and abuse of substances of vegetable origin. The use may be curative (vincristine and vinblastine, reserpine, ephedrine, aspirin, morphine, digoxin) or narcotic abuse (cocaine, morphine and cannabis), and misuse has made several plants endangered species (e.g. *Podophyllum hexandrum, Taxus baccata, Coptis teeta, Picrorhiza kurroa and Nardostachys jatamansi).* This overexploitation has resulted in depletion in germplasm resources, particularly in third world countries, and urgently warrants the development of alternative biotechnological methods for micropropagation, the study of seed and reproductive biology. It is estimated that approximately 1500 plant species in india are threatened including 124 endangered species. About 2,50,000 species of higher plants are yet to be investigated for pharmacological activity. Plant can be a source of effective remedies for Alzheimer's, parkinson's, epilepsy, migraine, arthritis and schizophrenia. Increased demand for natural drug has led to the domestication of several plants such as *Catharanthus roseus* and *Taxus baccata,* and several others (*Psoralea corylifolia, Carthamus tinctorius)* are being evaluated for agronomic traits. Improvements in isolation techniques to meet the demand for pharmacology, the generation of large of samples from correctly identified plants from the tropics for high-throughput screening, elaborate arrangements for preclinical (pharmacology, toxicology, pharmacokinetics and drug delivery) and clinical trials are required for drug development.

DEVELOPMENT IN HERBAL MEDICINE INDUSTRY WITH REFERENCE TO TRADE

There is great demand for herbal medicine in the developed as well as developing countries like India, because of their wide biological activities, higher safety of margin than the synthetic drugs and lesser costs. Medicinal plants play a great role in food supplements for care as well as in personal care of the mankind alongside the therapeutically active substances, thus medicinal plant based industry is a promising sector and enormous economic growth potential. Nutraceuticals (Health Food) are in great demand in the developed world particularly USA and Japan. Nutraceutical market in USA alone is about $ 80-250 billion, with a similar market size in Europe and Japanese sales worth $ 1.5 billion. Such huge markets have arisen because of the Dietary Supplement Health Education Act passed by USA in 1994, which permits unprecedented claims to be made about food or the dietary supplements ability about health benefits including prevention and treatment of diseases. This act has motivated pharma to include not only compounds isolated from fauna and flora but also herbal medicines as Nutraceuticals, which is unfortunate. The Indian herbal pharma companies also see this as a good opportunity and are marketing such products. However, the importance of medicinal plants in the national economy and their potential for the rapid growth of herbal products, perfumery and allied industry in India has been emphasized from time to time. New trends are emerging in the standardization of herbal raw materials whereby it is carried out to reflect the total content of phytoconstituents like polyphenols, which can be correlated with biological activity. The major traditional sector pharmas, namely Himalaya, Zandu, Dabur, Hamdard, Maharishi, etc, are Standardizing their herbal Formulations by Chromatography techniques like TLC/ HPTLC finger printing, etc.

HERBAL MEDICINE STANDARDIZATION

In indigenous/traditional systems of medicine, the drugs are primarily dispensed as water decoction or ethanolic extract. Fresh plant parts, juice or crude powder are a rarity rather than a rule. Thus medicinal plant parts should be authentic and free from harmful materials like pesticides, heavy metals, microbial or radioactive contamination, etc. The medicinal plant is subjected to a single solvent extraction once or repeatedly, or water decoction or as described in ancient texts. The extract should then be checked for indicated biological activity in experimental animal models. The bioactive extract should be standardized on the basis of active principle or major compounds along with fingerprints. The next important step is stabilization of the bioactive extract with a minimum shelf-life of over a year. The stabilized bioactive extract should undergo regulatory or limited safety studies in animals. Determination of the probable mode of action will explain the therapeutic profile. The safe and stable herbal extract may be marketed if its therapeutic use is well documented in indigenous systems of medicine, as also viewed by WHO. A limited clinical tribal to establish its therapeutic potential would promote clinical use. The herbal medicines developed in this mode should be dispensed as prescription drugs or even OTC products depending upon disease consideration and under no circumstances as health foods or nutraceuticals.

CHAPTER-2

LITERATURE REVIEW

LITERATURE REVIEW

PLANT DESCRIPTION

BOTANICAL CLASSIFICATION:

Domain	:	Eukaryota
Kingdom	:	Plantae
Sub kingdom	:	Viridiplantae
Class	:	Magnoliopsida
Sub class	:	Commelinidae
Order	:	Commelinales
Division	:	Magnoliophyta
Super division	:	Spermatophyta
Genus	:	*Commelina*
Species	:	*Benghalensis*
Family	:	Commelinaceae
Botanical name	:	*Commelina benghalensis L.*
Synonym	:	*Commelina mollis Jacq.*
		Commelina turbinata Vahl.
Common name	:	Benghal dayflower, Tropical spiderwort, Wandering jew.

VERNACULAR NAMES

Bengali	:	Kanchira, Jata-kanshira, Dholapata
English	:	Day flower
Hindi	:	Kanchara
Marathi	:	Kena
Malayalam	:	Adukkavettila, Vuzhaipadathi, Vazhaplaachi, Kanavazhai
Sanskrit	:	Kanchata
Tamil	:	Kanangkarai, Kanavazhiain

HABITAT AND DISTRIBUTION

Commelina benghalensis L. is a perennial herb native to tropical Asia and Africa. It is otherwise known as the paleotropics. *Commelina benghalensis L.* is often found in forest edges, road sides, cultivated fields, agricultural sites and home garden.

DESCRIPTION

Herbs perennial, stems mostly creeping, ascending distally, diffuse, numerous branched, up to 70 cm, sparsely pubescent. Leaf sheaths sparsely hirsute-ciliate; petiole distinct; leaf blade ovate, 3–7 × 1.5–3.5 cm, subglabrous. Involucral bracts borne opposite leaves, often several, aggregated at apex of branches, very shortly stalked, funnelform, 0.8–1.2 cm, sparsely hairy, proximal margins connate, apex acute or obtuse. Proximal branch of cincinni with

elongate peduncle and 1–3 exserted, infertile flowers, distal branch longer, with several fertile flowers. Sepals calyx 2 mm, membranous. Petals blue, 3–5 mm. Capsule ellipsoid, 4–6 mm, 3-valved; posterior valve 1-seeded or seedless, indehiscent; other 2 valves each 2-seeded, dehiscent. Seeds black, cylindric or semicylindric, ca. 2 mm, rugose, irregularly reticulate, truncate at 1 end. Fl. summer to autumn.

Wet places; near sea level up to 2300m. Anhui, Fujian, Guangdong, Guangxi, Guizhou, Hainan, Hebei, Henan, Hubei, Hunan, Jiangsu, Jiangxi, Shaanxi, Shandong, Sichuan, Taiwan, Yunnan, Zhejiang (tropical and subtropical Africa and Asia).

FLOWER

Cleistogamous flowers are formed in the smaller, funnel form involucral bracts on slender and creeping branches, which possess bladeless leaf sheaths and arise from the base of the plants. Flowers blue colour.

FRUITS (Capsule)

Capsules produced by such flowers are at the soil surface or in the soil, but only 1 or 2 valves are developed, each containing 1 or 2 seeds, which are larger than normal.

In all districts except in the wettest localities; Sea-level to 4000 ft. The lower nodes sometimes develop naked underground shoots bearing smaller white flowers which ripen large seeds underground, whereas perfect seeds are often not developed in the normal flowers. (**Gamble JS and Fischer CEC, 1967: Hong Deyuan and Robert A. DeFilipps, 2000**).

ETHNOCLAIM USES

LEAVES

Sandhya B *et al*., 2006, had investigated this plant used by Valaiyans an ethnic group residing in and around Piranmalai hills, Tamilnadu in combination with *Jasminum angustifolium* leaf used for the treatment of rabies.

Mahabub Nawaz AH Md *et al.,* 2009, had surveyed ethnobotanical information of traditional healers of Rajshahi district, Bangladesh, using paste of young leaves mixed with lime and a little amount of salt and massaged on the affected area twice daily in the morning and evening for external poisoning.

Gupta A *et al*., 2010, had investigated ethnopotential of medicinal herb is used for skin diseases is used to treat wounds.

LEAF AND ROOT

Mohammed Rahmatullah *et al.,* 2012, had surveyed this medicinal plant formulation of Kavirajes Faridpur and Rajbari districs, Bangladesh. Leaves of *Commelina benghalensis L*. are macerated with rhizomes of *Zingiber officinale* and applied to the hooves of cattle at the same time roots of *Commelina benghalensis L.* are tied around the throat of cattle for foot and mouth diseases in cattle.

WHOLE PLANT

Nadkarni K M, 1926, had reported the whole plant used as a demulcent, refrigerant, and laxative.

Lewu F B and Afolayan A J, 2009, had studied ethno medicinal importance of weedy species growing in SouthAfrica. They reported *Commelina benghalensis L*. traditionally used to treat infertility.

Mohammed Rahmatullah *et al*., 2010, had surveyed medicinal plant used by folk medicinal practitioners in ten districts of Bangladesh to treat leprosy.

Chaitanya *et al*., 2013, had reported ethnomedicinal uses of weedy species in Nilgiris, Tamilnadu. The Badagas of Nilgiris used this plant decoction internally to cure worm infection.

Kumar P *et al*., 2013, had studied medicinal floristic wealth at Aravalli Hills range in Khanak (Haryana), India. They reported *Commelina benghalensis L*. used for the treatment of burn, skin diseases and conjunctivitis.

Raphael Ranjit Marandi and John Britto S, 2015, had studied medicinal properties of edible weeds of crop fields and wild plants eaten by Oraon Tribals of Latehar District, Jharkhand. They reported that *Commelina benghalensis L*. young plant is cooked as a vegetables and used as a emollient, laxative, and demulcent. They also used for the treatment of leprosy, suppurative sores, snake bite, swelling, burns, antioxidant, cancer, ulcer, and skin diseases.

FLOWER

Jimmy Okello and Paul Ssegawa, 2007, had surveyed medicinal plants used by communities of Ngai subcounty, Apac district, Northen Uganda. Flowers are squeezed into the nose for the treatment of nasal blockade in children.

STEM

Muniappan Ayyanar and Savarimuthu Ignacimuthu, 2011, had surveyed this plants commonly used by Kani tribals of Tirunelveli hills of Western Ghats, India. They reported stem juice is topically applied for the treatment of wounds.

ROOT

Bhaskar VV and Patil HM, 2005, had reported tribals of Nandurbar district in Maharashtra, India using root powder admixed with equal amount of jaggary for the treatment of epilepsy.

Arshad Mehmood Abbasi *et al.,* 2013, had surveyed ethnobotanical appraisal and cultural values of medicinally important wild edible vegetables of lesser Himalayas-Pakistan. Dried roots of *Commelina benghalensis L*. grinded and powder is taken orally for epileps

Sheila Maregesi M *et al*., 2016, had studied plants traditionally used for the treatment of eye diseases in Kigoma district, Tanzania. They reported *Commelina benghalensis L*. sap and root decoction is warmed with camphor used as eye drops for eye ailments and conjuctivities.

PHYTOCHEMICAL REVIEW

LEAVES

Udaya Prakash NK *et al*., 2011, had studied phytochemical analysis of aqueous extract of *Commelina benghalensis L*. showed the presence of tannins, phlobatannins, saponins, flavonoids and absence of terpenoids, cardiac glycosides and steroids.

Bodke SS *et al*, 2012, had studied preliminary phytochemical analysis of weeds in Marathwada region. They reported *Commelina benghalensis L*. showed the presence of alkaloids, tannins, saponins, steroids and flavonoids.

Bibin Baby Augustine *et al*., 2013, had reported preliminary phytochemical analysis of hydroalcoholic extract of *Commelina benghalensis L*. It showed the presence of alkaloids, flavonoids, tannins, carbohydrates and saponins.

Prayaga Murty P *et al.,* 2013, had studied preliminary phytochemical screening of some weed species of Kadapa district, Andhra Pradesh, India. *Commelina benghalensis L*. showed the presence of alkaloids, saponins, tannins, steroid and flavonoids.

Kharade Amit S *et al*., 2013, had reported preliminary phytochemical investigation of Aqueous and Alcoholic extracts of *Commelina benghalensis L*. It showed the presence of alkaloids, carbohydrates, phytosterol, flavonoids, terpenoids, quinon and tannins.

Udaya Prakash NK *et al*, 2013, had studied phytochemical analysis of aqueous extract of *Commelina benghalensis L*. showed the presence of phlobatannins and saponins.

Chichioco Hernandez *et al*., 2014, had studied phytochemical analysis of methanolic extract of *Commelina benghalensis L*. showed the presence of terpenoids and flavonoids.

Krishna Satya A *et al*., 2016, had evaluated qualitative phytochemical analysis of chloroform and aqueous extracts of *Commelina benghalensis L*. Chloroform extract showed the presence of alkaloids, flavonoids, tannins and absence of phenol, terpenoids, saponins. Aqueous extract showed the presence of alkaloids, tannins and absence of phenol, flavonoids, terpenoids, saponins.

Sumithra D and Sumithra Purushothaman, 2017, had studied phytochemical profiling of ethanolic extract of *commelina benghalensis L.* by using Gas chromatography-Mass spectrometry (GC-MS). GC-MS analysis of *commelina benghalensis L.* revealed the presence of bioactive compounds such as 3-dodecene, 1-hexadeconol, 9-eicosene and tetratriacontane, Phenol 2,4 bis(1,1 dimethyl ethyl), hexadecen1 ol trans9, 9,10 anthracenedione, tetracosane, 1,4 benzene-dicarboxylic acid, bis (2ethylhexyl) ester, 13 docosenamide, tetracosane 11 decyl.

Sumithra.D and Sumithra Purushothaman, 2017, had investigated phytochemical screening of different (acetone, ethanol and water) extract of *Comelina benghalnsis L*. Aqueous extract showed the presence of carbohydrate, phlobatannins, flavonoid, saponin, tannin, volatile oil. Ethanol extract showed the presence of carbohydrate, phlobatannins, alkaloid, flavonoid, saponin, tannin, volatile oil, anthraquinone. Acetone extract showed the presence of carbohydrate, phlobatannins, alkaloid, flavonoid, saponin, volatile oil, anthraquinone.

LEAVES & STEMS

Sharmila Banu Ghani *et al*., 2016, had studied preliminary phytochemical screening of different extracts with solvents such as (aqueous, methanol, hexane, and carbontetrachloride) of *Commelina benghalensis L*. It showed the presence of phytochemical constituents such as alkaloids, protein, aminoacids, flavonoids, saponins, total phenols,and tannins and absence of anthraquinones, glycosides, steroids, and triterpenoids.

WHOLE PLANT

Armando *et al*., 2010, had evaluated preliminary phytochemical analysis of aqueous and alcoholic extracts of *Commelina benghalensis L.* It showed the presence of alkaloids, lactones, coumarins, triterpenes, steroids, resins, reducing agent, phenols, tannins, aminoacids, quinones, flavonoids and saponins.

Ibrahim *et al*., 2010, had performed thin layer chromatography and phytochemical analysis of *Commelina benghalensis L.* The phytochemical screening revealed the presence of phlobatannins, carbohydrates, tannins, glycosides, volatile oils, resins, balsams, flavonoids and saponins while terpenes, sterols, anthroquinones and phenols were absent. Thinlayer chromatography development revealed three spots for hexane extract, six spots for ethylacetate and five spots for methanol.

Ndam LM *et al*, 2014, had studied phytochemical screening of the bioactive compounds in twenty Cameroonian medicinal plants. They reported acetone extract of *Commelina benghalensis L*. showed the presence of steroids and flavonoids.

Balakrishnan CP and Jenifer Panneer, 2015, had studied phytochemical analysis of different extracts (Benzene, petroleum ether, chloroform, acetone, methanol, water) of *Commelina benghalensis L*. It showed the presence of bioactive compounds such as alkaloids, catechin, flavonoids, phenol, quinones, saponins, tannins, sugar, glycosides, protein, and aminoacids were present. Anthraquinone, coumarin, and xanthoprotein were absent.

Gurjar Himanshu PS *et al*., 2016, had studied preliminary phytochemical investigation of methanolic extraction of *Commelina benghalensis L*. It revealed the presence of different types of chemical constituents such as stigmasterol, alkaloids, carbohydrate, glycosides, flavonoids, aminoacids, and phenolic compounds.

Kaliyamoorthy Jayakumar, 2016, had investigated phytochemical screening of alcoholic extract of *Commelina benghalensis L*. It showed the presence of carbohydrate, phytosterols, alkaloids, flavonoids, and terpenoids, quinone, tannins.

TUBERS

Sharad Srivastava *et al*., 2016, had studied simultaneous reverse - phase HPLC determination of major antioxidant phenolics in methanolic extract of *Commelina benghalensis L*. Reverse – phase high performance liquid chromatography – photodiode array detection (RP-HPLC-PDA) method was developed for the separation, identification and quantification of bioactive phenolics. In this method five phenolics protocatechuic acid (0.033%), vanillic acid (0.262%), ferulic acid (0.365%), apigenin (0.126%) and kaempferol (0.544%) were quantified in linearity range of 0.2-1.0 µg with correlation coefficient of more than 0.9949. Antioxidant activity was determined by DPPH Radical scavenging activity. The methanolic extract shows the inhibition range from 24.45 to 68.75% at 0.02-0.12 mg Ml^{-1} in comparison with standard ascorbic acid, quercetin and rutin.

PHARMACOLOGICAL REVIEW

LEAVES

ANTIMICROBIAL ACTIVITY

Mukesh Chandra Sharma and Smita Sharma, 2010, had investigated antimicrobial activity of different (aqueous, hexane, chloroform, methanol) extracts of *Ixora coccinea L*. and *Commelina benghalensis L*. on Gram-Positive and Gram-Negative microorganism by agar well diffusion method. The extract concentration of 100% exhibit high antimicrobial activity against *E.coli* with modest activity against *S.typhi, S.aureaus*. Methanol extract showed broad spectrum activity in comparision with amoxicillin and gatifloxcin.

ANTIMICROBIAL ACTIVITY

Gothandam KM *et al*., 2010, had studied antimicrobial properties of methanolic extract of few medicinal plants by disc diffusion method. *Commelina benghalensis L*. showed the antimicrobial activity against Gram positive and Gram negative organism.

ANTIBACTERIAL ACTIVITY

Rajesh F. Udgirkar *et al*., 2012, had reported aqueous extract of *Commelina benghalensis L*. possessing antibacterial activity determined by agar diffusion method.

ANTI – INFLAMMATORY ACTIVITY

Bibin Baby Augustine *et al*., 2013, had reported anti-inflammatory and toxicity effect evaluation of hydroethanolic extract of *Commelina benghalensis L*. on female rats. Hydroethanolic extracts of *Commelina benghalensis L*. did not show any toxic reaction in female rats in acute and subacute toxicity test at a dose level of 200 and 400 mg/kg. Anti-inflammatory activity was studied by using carrageenan induced rat paw edema, cotton pellet granuloma and

xylene induced ear edema model at two different doses (200 and 400 mg/kg b.w). Hydroethanolic extract of *Commelina benghalensis L*. exhibited significant Anti-Inflammatory activity in carrageenan induced rat paw edema, at a dose level of 400 mg/kg. cotton pellet granuloma and xylene induced ear edema model at a dose level of 200 and 400 mg/kg of bodyweight shows significant Anti-inflammatory activity as compared to the control group with indomethacin.

ANTIPLASMODIAL ACTIVITY

Ngo Bum E, Ngo Yebga J, Njan Nloga AM, 2014, had studied antiplasmodial effect of aqueous leaf extract of *Commelina benghalensis L*. and bark of *Steganotaenia araliacea* on the Human populationin Ngaoundere (Cameroon). The combination of *Commelina benghalensis L.* and *Steganotaenia araliacea* extract mixture was given to patient for ten days. Antiplasmodial effect was assessed by frequencies and the parasites densities of plasmodium species. Treatment of patient with mixture of the extracts revealed a progressive reduction of infestation up to a total dispiriting of the parasites after ten days and shows significant antiplasmodial activity in comparision with Amodiaquine.

15-LIPOOXYGENASE INHIBITION ACTIVITY

Chichioco Hernandez *et al*., 2014, had evaluated 15-Lipooxygenase inhibition of methanolic extract of *Commelina benghalensis L*., *Tradescantia fluminensis,* and *Tradescantia zebrina* by 15-Lipooxygenase inhibitory assay. The inhibitory activity was evaluated by using a spectrophotometric assay by observing the increase in absorbance at 234 nm due to the formation of the product 13 – hydro peroxy octadecadienoic acid. All the methanolic extracts significantly inhibited the action of 15 – lipooxygenase at a concentration of 0.2 µg/ml.

THROMBOLYTIC AND CYTOTOXIC ACTIVITY

Abul Hasanat *et al*., 2015, had investigated thrombolytic and cytotoxic activity of methanolic extract of *Commelina benghalensis L*. The cytotoxicity had been assessed by Brine shrimp lethality bioassay and also thrombolytic activity had been assessed by thrombolytic impact with individual blood. The Brine shrimp lethality bioassay result was (LC50 = 278.69 µg/ml) compared with standard vincristine sulphate (LC50 = 0.512 µg/ml). It has significant thrombolytic activity (40.94%) compared with standard streptokinase (75%).

ANTIBACTERIAL ACTIVITY

Joy Prabhu H and Johnson I, 2015, had investigated antibacterial activity of silver nanoparticles synthesized from aqueous extract of *Commelina benghalensis L*. by disc diffusion method. The antimicrobial activity of silver nanoparticles was investigated against *escherichia coli, klebsiella pneumonia, vibrio cholorae, salmonella typhi, and shigella sonnei* by disc diffusion method. These results suggest that silver nanoparticles can be used as effective growth inhibitor in various microorganism, making them applicable to diverse medical system and antimicrobial control system.

ANTIDIARRHOEAL AND ANTHELMINTIC ACTIVITY

Mohammad Mamun Ur Rashid *et al*., 2016, had studied antidiarrhoeal and anthelmintic activity on methanolic extract of *Commelina benghalensis L*. Antidiarrhoeal activity was studied by invivo method such as castor oil induced diarrhea, castor oil induced enteropooling and gastrointestinal motility test in Swiss albino mice. Methanol extract at 200 and 400 mg/kg exhibited significant dose dependent antidiarrhoeal activity in comparision with standard drug loperamide. Anthelmintic activity was determined by invitro anthelmintic assay in aquarium worm Tubifex tubifex. Methanol extract showed dose dependent Anthelmintic activity when compared with Levamisole used as standard.

ANTICOAGULANT ACTIVITY

Krishna Satya A *et al*., 2016, had studied efficacy of medicinal plants against the lethality of Naja Naja snake envenomation. Inhibiting property of (toxic) lethal factors like PLA_2, coagulant activity, proteolytic activity and aceylcholine esterase activity of venom were studied. Aqueous extract of *Commelina benghalensis L*. inhibits the serum coagulation of the venom. Aqueous extract of *Commelina benghalensis L*. in PLA_2 activity inhibition is 59%. The coagulant factors in the venom of Naja Naja were inhibited by aqueous extract of *Commelina benghalensis L*.

. **ANTIBACTERIAL ACTIVITY**

Sumithra D and Dr. Sumithra Purushothaman, 2017, had investigated antibacterial activity of different (acetone, ethanol, water) extracts of *Commelina benghalensis L*. by agar well diffusion method. It showed the antibacterial activity against *escherichia coli, bacillus subtilis, staphylococcus aureus, enterococcus faecalis* while no inhibitory activity against *klepseilla pneumonia*.

AERIAL PART

SEDATIVE AND ANXIOLYTIC ACTIVITY

Shafiqur Rahman *et al*., 2009, had investigated different soluble fraction (chloroform, petroleum ether, n-butanol, hydromethanol) of the *Commelina benghalensis L*. showed Sedative and Anxiolytic properties. Sedative property was determined by Rodent behavioural model such as hole cross, open field and Thiopental sodium induced sleeping time in mice. chloroform and petroleum ether fraction showed significant dose dependent sedative activity in comparision with Diazepam. Anxiolytic property was determined by elevated plus-maze (EPM) Test in mice. chloroform and petroleum ether fraction showed significant dose dependent anxiolytic activity in

comparision with diazepam. Hydromethanol and n-butanol fraction are not found to be statistically significant.

ANALGESIC ACTIVITY

Mazumder MEH *et al*., 2010, had evaluated different fraction (petroleum ether, chloroform, n-butanol, hydromethanol) of the *Commelina benghalensis L*. extracts exhibited Analgesic activity. Peripherally acting analgesic activity was determined by Aceticacid-induced writhing test in mice. The different fraction of extract showed significant dose dependent analgesic activity in comparison with diclofenac sodium. Centrally acting analgesic activity was determined by hot plate and tail immersion method in mice. The different fraction of extract exhibited significant analgesic activity in comparison with standard drug Nalbuphine. All fraction at the dose of 200 and 400 mg/kg of bodyweight showed significant analgesic action in a dose dependent manner in the tested models.

ANTIOXIDANT ACTIVITY

Anusuya *et al*., 2012, had evaluated antioxidant and free radical scavenging effect of different extracts (Acetone, Methanol, Water) of *Commelina benghalensis L*. by various invitro assays. All the extracts contained considerable levels of total phenolics, tannins, flavonoids, and vitamin c content and also exhibited increasing reducing activity with increasing concentration.

ANXIOLYTIC ACTIVITY

Madhuri D. Bhujbal *et al*., 2012, had reported anxiolytic properties of the four different soluble fraction (chloroform, petroleum ether, n-butanol, hydromethanol) of the *Commelina benghalensis L*. by rodent behavioural model. maximum effect was shown by chloroform and petroleum ether fraction compared to the reference standard Diazepam. chloroform and petroleum ether soluble fraction has significant anxiolytic effect.

ANTIOXIDANT ACTIVITY

Nizam Uddin *et al*., 2013, had studied DPPH scavenging assay of eighty four Bangladeshi medicinal plants. Chloroform, petroleum ether, n-butanol, hydromethanol extracts of *Commelina benghalensis L.* are assayed by DPPH scavenging assay. It showed excellent DPPH scavenging property (90% or more) in comparision with ascorbic acid and BHT (Butylated Hydroxy Toluene). petroleum ether fraction shows good scavenging property.

CYTOTOXIC ACTIVITY

Amina Khatun *et al*., 2014, had studied cytotoxicity potential of methanolic extract of *Commelina benghalensis L*. by brine shrimp lethality bioassay. It showed the quite high cytotoxicity LC_{50} ranging from 21 to 115 µg/mL.

WHOLE PLANT

ANTIBACTERIAL ACTIVITY

Parekh J and Chanda S, 2007, had studied invitro screening of antibacterial activity of aqueous and alcoholic extracts of *Commelina benghalensis L*. against selected pathogens from enterobacteriaceae. Antibacterial assay was performed by agar disc diffusion method for aqueous extract and agar well diffusion method for alcoholic extract. It showed the zone of inhibition in *Klebsiella pneumonia*e.

ANTIMICROBIAL ACTIVITY

Armando *et al*., 2010, had reported antimicrobial evaluation of aqueous and ethanolic extract of *Commelina benghalensis L*. by Agar well diffusion method. Antimicrobial assay was performed on *staphylococcus aureus, candida albicans* (isolated from patient), and *Escherichia coli*. Gentamycin for the bacteria and Nystatin for the fungai were used as a control antibiotics. The ethanol extract are superior in performance compared to the aqueous extract. In both

ethanolic extract activity against *candida albicans* > *escherichia coli* > *staphylococcus aureus*. Aqueous extract showed virtually no activity against the *staphylococcus aureus, escherichia coli* and *candida albicans* strains.

ANTIBACTERIAL ACTIVITY

Mohammad AA Khan *et al*., 2011, had studied antibacterial activity of different extracts (Ethanolic, Petroleum etheric, Diethyl etheric, Methanolic, Aqueous) of *Commelina benghalensis L*. by Agar disc diffusion method. Minimum inhibitory concentration was determined by micro dilution method. Antimicrobial activity of the extracts at two different concentration of 250 and 500 µl/discs were compared with those of positive control such as Azithromycin and Tetracycline at the dose of 30 µg/disc. The extract possess maximum potency against infectious pathogens such as *staphylococcus saprophyticus, staphylococcus aureus, enterococcus faecalis, staphylococcus pyogenes, streptococcus agalactiae, salmonella typhi, Escherichia coli, shigella boydii, shigella dysenteriae* and *pseudomonas aeruginosa*. It showed significant minimum inhibitory concentration.

NEPHROPROTECTIVE ACTIVITY

Kokilavani.P, Achiraman.S, Pandilakshmi.P, 2015, had investigated protective and curative effect of Aqueous extract of *Commelina benghalensis L*. and *Cissus quadrangularis* against quinalphos induced oxidative stress in swiss albino mice kidney tissue. Raising the level of serum urea, uric acid, and creatinine confirmed the nephrotoxicity induced by quinalphos. Significant increasing of creatinine level indicate the kidney damage. Protective effect of nephrotic damage was determined by serum biochemical analysis and histomorphological analysis of kidney.*Cissus quadrangularis* showed better protection compared to the *Commelina benghalensis L*. *Commelina benghalensis L.* and *Cissus quadrangularis* aqueous extract showed significante protection of nephrotic cell damage from oxidative stress caused by quinalphos.

ANTIDIABETIC ACTIVITY

Gurjar Himanshu PS *et al*., 2016, had studied antidiabetic activity of methanolic extract of *Commelina benghalensis L*. in male albino rat by Alloxan induced diabetes mellitus model. The methanolic exract of *Commelina benghalensis L*. (100,200,400 mg/kg i.p) has showed significant Antidiabetic activity in Alloxan induced diabetic rat in comparison with glibenclamide. It also significantly reduces the elevated level of blood cholesterol and triglyceride. The extract significantly improved the Alloxan induced reduction of blood protein to normal value.

ANTINOCICEPTIVE ACTIVITY

Mohammad Shah Hafez Kabir *et al*., 2016, had investigated antinociceptive action of methanolic extract of *Commelina benghalensis L*. in mice by aceticacid-induced writhing test and formalin induced licking test. The methanolic extract exhibited significant dose dependent antinociceptive activity in comparision with diclofenac sodium.

STEM

ANTICANCER ACTIVITY

Leseilane J.Mampuru *et al*., 2008, had Investigated Alteration of Bax-to-Bcl-2 ratio modulates the anticancer activity of methanolic extract of *Commelina benghalensis L*. in Jurkat T cells. Jurkat T cells were exposed to different concentration (0 – 600 µg/ml) of the crude methanolic extract of *Commelina benghalensis L*. to evaluate their growth inhibitory and apoptosis inducing effects. The extract elicited a dose and time dependent inhibition of cell proliferation, followed by a concomitant decrease in cell viability. The observed cytotoxicity was linked to the induction of apoptosis as determined by morphological and biochemical features known to be associated with the advent of apoptosis. Real time quantitative RT-PCR analysis of apoptosis regulatory genes and western blot analyses of Bax, Bcl-2 and p53 exhibited aberrant

expression profiles of these genes under various treatment condition. They reported crude methanolic extract of *Commelina benghalensis L*. contains bioactive compounds that may be beneficial in the treatment of malignant growths, and that this antineoplastic activity is a consequence of dysregulated expression of apoptosis responsive genes.

Matlou Phineas Mokgotho *et al*, 2014, had evaluated subfraction (n-hexane (F1) and dichloromethane (F2)) of acetone extracts of *Commelina benghalensis L*. induced apoptosis and cell cycle arrest in jurkat T cells. After treatment of Jurkat T cells with these subfractions, cell proliferation, viability, and apoptosis were determined by using a haemocytometer, the trypan blue dye exclusion assay, and Hoechst 33258 staining respectively. Cell division cycle distribution profile were analysed using an Epics alba flow cytometer and the expression of cell division cycle regulatory genes was analysed using RT-PCR, while immuno reactive proteins were detected on western blots. The F1 and F2 fraction inhibited the proliferation and viability of jurkat T cells in a concentration and time dependent manner. Both fractions induced a G1/S interphase arrest of the cell division cycle of jurkat T cells.

WOUND HEALING ACTIVITY

Gulzar Alam *et al*., 2011, had reported *Commelina benghalensis L.* juice is applied for healing of wounds.

ROOT

HEPATOPROTECTIVE ACTIVITY

Sambrekar SN *et al*., 2009, had studied hepatoprotective activity of various root extracts of *Commelina benghalensis L*. by paracetamol induced liver damage model in Wistar rats. Liver damage was produced by paracetamol. Aqueous as well as alcoholic extract showed significant hepatoprotective activity and efficacy of alcoholic extract was almost comparable to that of N-Acetyl 1-Cystine.

Sambrekar Sudhir N, Patil Suhas A *et al*., 2013, had investigated alcoholic and aqueous extract of *Commelina benghalensis L*. on ethanol induced acute hepatotoxicity in male wistar rats showed possible mechanism and hepatoprotective effect. Hepatoprotective effect was determined by invivo hepatotoxic parameter including serum transaminase (AST and ALT), ALP, bilirubin, protein, lipid profile (cholesterol, triglyceride, VLDL and HDL) and level of antioxidant together with histopathological examination. Liv[52] was used as a reference hepatoprotective agent. Phenobarbitone induced sleeping time study was carried out to verify the effect on microsomal enzymes. Histopathological observation confirmed the beneficial roles of MF against Ethanol induced liver injury in rats. Possible mechanism may involve their antioxidant activity.

ANALGESIC AND ANTI-INFLAMMATORY ACTIVITY

Sanjib Saha *et al*., 2014, had evaluated ethanolic extract of *Commelina benghalensis L.* showed Analgesic and Anti-inflammatory activity. Peripherally acting analgesic activity was determined by Acetic acid induced writhing test in swiss albino mice and centrally acting analgesic activity was determined by hot plate and tail flick test in mice. In aceticacid induced writhing test it showed significant analgesic activity in comparision with diclofenac sodium. In Hotplate and Tail flick test it showed significant analgesic activity in comparision with Morphine. Anti-inflammatory activity was determined by carrageenan induced paw edema in mice. The ethanol extract exhibited significant dose dependent anti-inflammatory activity in comparision with Indomethacin.

INFLORESCENCE

Baljinder Singh *et al*., 2011, had reported *Commelina benghalensis L*. used for eye diseases.

CHAPTER-3

AIM AND OBJECTIVE

AIM AND OBJECTIVES

AIM

The aim of present research is to investigate the Pharmacognostical, Phytochemical studies including Isolation of Lutein and its Sun Protection Factor, Invitro Anti-Inflammatory, Anti-Arthritic and Anti-oxidant activity of *Commelina benghalensis L.* (Commelinaceae).

OBJECTIVE

The present work has been planned to carry out the

PHARMACOGNOSTICAL STUDIES

- Authentification and collection of plant
- Macroscopy of the leaf
- Microscopy of the leaf
- Powder microscopy includes its identification of character
- Behavioural characters with different reagents

Physiochemical Parameters

- Foreign matter
- Loss on drying (LOD)
- Extractive value with various reagent
- Ash values
- Total solids

PHYTOCHEMICAL STUDIES

- Preparation of Hydroalcohol Extract
    - **Qualitative analysis**
        - Preliminary Phytochemical Screening
        - Identification of R_f value by TLC method

- **Quantitative Estimation of Phytoconstituents**
    - Tannic acid
    - Gallic acid
    - Rutin in terms of its equivalents
    - Determination of Chlorophyll "a", Chlorophyll "b", Total chlorophyll and Total carotenoids.

ISOLATION OF LUTEIN

PHARMACOLOGICAL STUDIES

- **Determination of Sun Protection Factor of Isolated Lutein From *Commelina benghalensis L.***
- **Invitro Anti-Inflammatory Activity Screening By Membrane Stabilization Study**
- **Invitro Antiarthritic Activity By Protein Denaturation Method**
- **In vitro Antioxidant Activity**
    - Hydrogen peroxide scavenging activity
    - Reducing power assay
    - Total antioxidant activity

CHAPTER-4

MATERIALS AND METHODS

MATERIALS AND METHODS

PLANT COLLECTION & AUTHENTIFICATION

Fresh leaf of *Commelina benghalensis L.* were collected from Madurai Medical College, Madurai (DT), during the month of August- 2017 and was authenticated by Dr. D. Stephen, M.Sc., Ph.D., Assistant professor, Department of Botany, American College, Madurai-20. The herbarium of this specimen was kept in the department for further reference.

PART A

PHARMACOGNOSTICAL STUDIES

Morphological and micro morphological examination and characterization of medicinal plants have always been accorded due credentials in the pharmacognostical studies. Botanical identity of the plants is an essential prerequisite for undertaking the analysis of medicinal properties of any plant. A researcher may succeed in getting a new compound or may find many useful pharmacological active properties in the plant. If the botanical identity of the plant happens to be dubious or erratic, the entire work on the plant becomes invalid. Thus it is needless to stress the botanical identity of the crude drug is the threshold in the processes of pharmacological investigations. The researchers should be equipped with all possible diagnostic parameters of the plant on which the researchers plan to work.

MORPHOLOGICAL STUDIES OF *Commelina benghalensis L.*

Leaves were studied seperately for its morphological characters by organoleptic test.

MICROSCOPICAL STUDIES OF *Commelina benghalensis L.*

Fresh leaves were selected for the microscopical parameters by using microscope.

COLLECTION OF SPECIMEN

Care was taken to select healthy plants and for normal organs. Leaf, Petiole specimens were collected from a healthy plant by making a cut with petioles. The materials were cut into pieces and immediately immersed in fixative fluid FAA (Formalin – 5ml + Acetic acid – 5ml +70% Ethyl alcohol – 90ml).

DEHYDRATION

After 24 hours of fixing, the specimens were dehydrated with graded series of ethyl alcohol and tertiary-butyl alcohol (Sass, 1940). The specimen is kept is in each grade of the fluid for about 6 hrs. Every time the fluid is decanted and immediately the specimen were flooded with next grade of fluid.

INFILTRATION WITH PARAFFIN WAX

After dehydration, the shavings of paraffin wax were added to the vial containing the plant material with pure TBA. The paraffin shavings are added every 30mts at about 40-45ºC four or five times. Then the vials were filled with wax without damaging the tissues. The vial filled with wax is kept open in warm condition to evaporate all TBA, leaving the specimen in pure molten wax. The specimen filled with pure molten wax for 2 or 3 times by decanting the old wax every time.

CASTING TO MOLD

A boat made out of chart board, by folding the margin, is used to prepare a mold of wax containing specimens. The paraffin along with the leaf and petiole specimen was poured into the boat. With the help of heated needles, the specimen were arranged in parallel rows with enough space in between the specimens. The block was then immersed in chilled water and allowed to cool for few hours.

SECTIONING

The paraffin embedded specimens were sectioned with the help of microtome**.** The thickness of the sections was 10-12µm. Dewaxing of the sections was by customary procedure. The sections were stained with **Toluidine blue** as per the method published by O'Brien *et al* (1964). Since toluidine blue is a poly chromatic strain, the straining results were remarkably good and some **cytochemical reactions** were also obtained. The dye rendered pink colour to the **cellulose** walls, blue to the lignified cells, dark green to **suberin**, violet to the **mucilage**, blue to the **protein** bodies etc. Where ever necessary sections were also stained with **safranin** and **fast-green** and potassium iodide (for starch). For studying the stomatal morphology, venation patttern and trichome distribution, **paradermal sections** (sections taken parallel to the surface of leaf as well as **clearing** of leaf with 5% sodium hydroxide or epidermal peeling by partial maceration employing Jeffrey's maceration fluid (Sass, 1940) were prepared. Glycerin mounted temporary preparations were made for macerated/cleared materials. Powdered materials of different parts were cleared with sodium hydroxide and mounted in glycerin medium after staining. Different cell components were studied and measured.

PHOTOMICROGRAPHS

Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon labphot 2 Microscopic unit. For normal observations bright field was used. For the study of crystals, starch grains and lignified cells, polarized light were employed. Since these structures have birefringent property, under polarized light they appear bright against dark background. Magnifigations of the figures are indicated by the scalebars. (Johansen DA, 1940).

PREPARATION OF LEAF POWDER

The leaves were collected and shade dried. It was powdered in a mixer. The coarse powder was sieved and was stored in a well closed container.

POWDER MICROSCOPY

The coarse powder was treated with routine reagents to identify the diagnostic features of the plant..

QUANTITATIVE MICROSCOPY OF *Commelina benghalensis L.*

Fresh leaves of *Commelina benghalensis L.* was subjected to microscopical study includes in stomatal number, stomatal index were determined on fresh leaves using standard procedure. **(Wallis TE. 1953, Wallis TE, 1965).**

STOMATAL INDEX

It is the percentage, which the numbers of stomata from the total number of epidermal cells, each stoma being counted as one cell.

I. **Stomatal index = S/S+E x 100**

Where, S = Number of stomata per unit area

E = Number of epidermal cells in the same unit area

DETERMINATION OF STOMATAL INDEX

The procedure adopted in the determinations of stomatal number was observed under high power (45 X).The epidermal cells and the stomata were counted. From these values the stomatal index was calculated using the above formula. The results obtained are presented in **Table: 2**

PHYSIO-CHEMICAL PARAMETERS

The powder was subjected to physiochemical parameters such as foreign organic matter, loss on drying, ash values and extractive values with different solvents in increasing order of polarity, volatile oil, and total solids. The procedure was adapted as per **WHO guidelines 1998, and James 1995.**

DETERMINATION OF FOREIGN ORGANIC MATTER

PROCEDURE

An accurately weighed 100g of air dried coarse drug and spread out in a thin layer. The sample drug was inspected with the unaided eye or with the use of 6x lens and the foreign organic matter was separated manually as completely as possible and weighed. The percentage of foreign organic matter was calculated with reference to the weight of the drug taken.

DETERMINATION OF MOISTURE CONTENT (LOSS ON DRYING)

PROCEDURE

An accurately weighed 10 g of coarsely powdered drug was placed in a tarred evaporating dish. Then the dish was dried at 105^{o}C for 5 h and weighed. The drying and weighing was continued at one hour intervals until the difference between the two successive weighing is not more than 0.25 %. The loss on drying was calculated with reference to the amount of powder taken.

DETERMINATION OF SWELLING INDEX

The swelling index is the volume in ml taken up by the swelling of 1g of plant material under specified conditions.

PROCEDURE

About 1g of the crude powder was weighed and transferred to the 25ml of glass stoppered measuring cylinder of 25ml of water and was shaken thoroughly for every10 min for 1 hour. It was allowed to stand at room temperature for 3 hours. The volume in ml occupied by the plant material including and sticky mucilage. The weight was calculated with refer to the dried weight.

DETERMINATION OF EXTRACTIVE VALUES

PROCEDURE

An accurately weighed 5 g of the air dried coarsely powdered drug was macerated with 100mL of various solvents of increasing order of polarity (petroleum ether, benzene, chloroform, ethyl acetate, ethanol, methanol and water) in a closed flask for 24 h, shaking frequently during the first 6 h and allowed to stand for 18 h.

Thereafter filtered rapidly, taking precautions against loss of ethanol. Then evaporate 25 mL of the filtrate to dryness in a tarred flat bottomed shallow dish dry at 105^{o} C and weighed.

DETERMINATION OF ASH VALUES

ASH CONTENT

The residue remaining after incineration is the ash content of crude drug, which simply represents inorganic salts naturally occurring in the drug or adhering to it or deliberately added to it as a form of adulteration.

DETERMINATION OF TOTAL ASH

PROCEDURE

An accurately weighed 3 g of air dried coarsely powdered drug was taken in a tarred silica crucible and incinerated at a temperature not exceeding 450^{o} C, until free from carbon then allowed to cool and weighed. The percentage of ash was calculated with reference to the air dried drug.

DETERMINATION OF ACID INSOLUBLE ASH

PROCEDURE

The total ash obtained from the previous procedure was mixed with 25 ml of 2 M hydrochloric acid and boiled for 5 min in a water bath, and then the insoluble matter was collected in an ash less filter paper and washed with hot water, dried and ignited for 15 min at a temperature not exceeding 450^{o} C, cooled in desiccators and weighed. The percentage of acid insoluble ash was calculated with reference to the air dried drug.

DETERMINATION OF WATER SOLUBLE ASH

PROCEDURE

The total ash obtained from the previous procedure was mixed with 25 ml of water and boiled for 5 min in a water bath, and then the insoluble matter was collected in an ash less filter paper and washed with hot water, dried and ignited for 15 min at a temperature not exceeding 450^{o} C, cooled in desiccators and weighed. The insoluble matter was subtracted from the weight of the total ash; the difference in weight represents the water soluble ash. The percentage of water soluble ash was calculated with reference to the air dried drug.

DETERMINATION OF TOTAL SOLIDS

PROCEDURE

Preparation of evaporating dishes–If volatile solids are to be measured, ignite clean evaporating dishes and watch glasses at 550ºC for 1 hour in a muffle furnace, heat dishes and watch glasses at 103ºC to 105ºC for 1 hour in an oven. Cool and store the dried equipment in a dessicator. Weigh each dish and watch glass prior to use (record combined weight as "W_{dish}").

$$\textbf{Total solids} = W_{Total} - W_{Dish} / W_{Sample} - W_{Dish} \times 100$$

The results of physio-chemical parameters were tabulated in **Table: 3**

Behavioural characters of the *Commelina benghalensis L.* (Leaf- crude powder)

Crude powder of *Commelina benghalensis L.* treated with various chemical reagents (Water, Con. HCL, Con.H_2SO_4, Con HNO_3, CH_3COOH, Con. HCL+ Water, Con.H_2SO_4+ Water, Con HNO_3+ Water, CH_3COOH + Water, Aqueous NaOH, Aqueous $Fecl_3$) and their behavioural characters are tabulated in **Table: 4**

PREPARATION OF HYDROALCOHOLIC EXTRACT OF *Commelina benghalensis L.*

PROCEDURE

The shade dried and coarsely powdered leaf of *Commelina benghalensis L.* (Leaf) was defatted with petroleum ether (60-80˚c). The residue was dried and extracted with hydroalcohol

(70%) by Maceration until the complete extract of the material and filtered. The extract was concentrated under reduced pressure to obtain a solid residue (dark brown).

The above extract was subjected to physical analysis such as colour, consistency, wt/ ml, refractive index and brix. The results obtained are presented in **Table: 5**

PART B

QUALITATIVE AND QUANTITATIVE ANALYSIS

The hydroalcoholic extract was subjected to qualitative and quantitative analysis. Qualitative analysis includes phytochemical screening of secondary metabolites such as flavonoids, carbohydrates, alkaloids, glycosides, sterols, tannin, protein, aminoacids, carotenoids, volatile oil, quinone, terpenoids, phenolic content and Thin layer chromatography of the extract were determined. Quantitative analysis includes estimation of total tannin, total gallic acid, total flavonoid contents in terms of total tannic acid equivalent, total gallic acid equivalent, total flavonoids equivalent (rutin) and total carotenoid and total chlorophyll content and extract were determined.

QUALITATIVE ANALYSIS

PRELIMINARY PHYTOCHEMICAL SCREENING

Hydroalcoholic extract of *Commelina benghalensis L.* (Leaf) was subjected to qualitative chemical analysis. The various chemical tests were performed on this extract and aqueous extract for the identification of flavonoids, phenolic compounds, alkaloids, glycosides, carbohydrates, carotenoids, proteins, tannin, aminoacids, sterols as per Harborne 1998.

TEST FOR ALKALOIDS

About 2 gm of the powdered material was mixed with 1gm of calcium hydroxide and 5 mL of water into a smooth paste and set aside for 5 minutes. It was then evaporated to dryness in a porcelain dish on a water bath. To this 200 mL of chloroform was added, mixed well and refluxed for half an hour on a water bath. Then it was filtered and the chloroform was evaporated. To this 5 mL of dilute hydrochloric acid was added followed by 2 mL of each of the following reagents.

MAYER'S TEST:

A small quantity of the extract was treated with Mayer's reagent. Cream colour precipitate indicates the presence of alkaloids.

DRAGENDORFF'S TEST:

A small quantity of the extract was treated with Dragendorff's reagent. Orange brown precipitate indicates the presence of alkaloids.

WAGNER'S TEST:

A small quantity of extract was treated with Wagner's reagent. Reddish brown precipitate indicates the presence of alkaloids.

HAGER'S TEST:

A small quantity of extract was treated with Hager's reagent. Yellow precipitate indicates the presence of alkaloids.

TEST FOR PURINE GROUP (MUREXIDE TEST)

The residue obtained after the evaporation of chloroform was treated with 1mL of hydrochloric acid in a porcelain dish and 0.1 gm of Potassium chlorate was added and evaporated to dryness on water bath. Then the residue was exposed to the vapour of dilute ammonia solution. No purple Colour was obtained indicating the absence of purine group of alkaloids.

TEST FOR INDOLE

To the test solution, add acetic acid and trace amount of anhydrous $FeCl_3$, under – lay /H_2SO_4 intense blue at interface.

TEST FOR QUINOLINE (Thalleioquin Test)

To the extract, add 1 drop of dilute sulphuric acid and 1ml of water. Add bromine water drop wise till the solution acquires permanent yellow colour and add 1mL of dilute ammonia solution, emerald green colour is produced. The powdered drug when heated with glacial acetic acid in dry test tube, evolves red fumes, which condense in the top portion of the tube. The bark, when moistened with sulphuric acid and observed under ultraviolet light shows a blue fluorescence due to the methoxy group of quinine and quinidine.

TEST FOR CARBOHYDRATES

MOLISCH'S TEST

The extract of the powdered drug was treated with 2-3 drops of 1% alcoholic α naphthol and 2mL of concentrated sulphuric acid was added along the sides of the test tube. A purple colour indicating the presence of carbohydrates.

FEHLING'S TEST

The extract of the powdered leaf was treated with Fehling's solution I and II and heated on a boiling water bath for half an hour. Red precipitate was obtained indicating the presence of free reducing sugars.

BENEDICT'S TEST

The extract of the powdered leaf was treated with equal volume of Benedict's reagent. A red precipitate was formed indicating the presence of reducing sugar.

TEST FOR ANTHRAQUINONE GLYCOSIDES

BORNTRAGER'S TEST

The powdered drug was boiled with dilute sulphuric acid, filtered and to the filtrate benzene was added and shaken well. The organic layer was separated to which ammonia solution was added slowly. No pink colour was observed in ammoniacal layer showing the presence of anthraquinone glycosides.

MODIFIED BORNTRAGER'S TEST

About 0.1 g of the powdered drug was boiled for 2 minutes with dil.HCl and few drops of $FeCl_3$ solution, filtered while hot and cooled. The filtrate was then extracted with benzene and the benzene layer was separated. Equal volume of dil.NH_3 solution was added to the benzene extract. No pink colour was observed in ammoniacal layer showing the presence of glycosides.

TEST FOR CARDIAC GLYCOSIDES (FOR DEOXYSUGAR)

KELLER KILIANI TEST

About 1 g of the powdered leaf was boiled with 10 mL of 70 % alcohol for 2 minutes, cooled and filtered. To the filtrate 10 mL of water and 5 drops of solution of lead subacetate were added and filtered, evaporated to dryness. The residue was dissolved in 3 mL of glacial acetic acid. To these 2 drops of ferric chloride solution was added. Then 3 mL of concentrated H_2SO_4 was added to the sides of the test tube carefully and observed. No reddish brown layer was observed indicating the absence of deoxysugars.

RAYMOND TEST

Test solution treated with dinitrobenzene in hot methanolic alkali gives violet colour.

LEGAL'S TEST

Test solution when treated with pyridine made alkaline by sodium nitro prusside solution gives pink to red colour.

TEST FOR CYANOGENETIC GLYCOSIDES

Small quantity of the powder was placed in a stoppered conical flask with just sufficient water, to cover it. A sodium picrate paper strip was inserted through the stopper so that it was suspended in the flask and it was set aside for 2 hours in a warm place. Brick red colour was produced on the paper indicating the presence of cyanogenetic glycosides.

TEST FOR COUMARIN GLYCOSIDES

WITH AMMONIA

Take a drop of ammonia on a filter paper; to this add a drop of aqueous extract of leaves. Development of fluorescence shows positive test for coumarins.

WITH HYDROXYLAMINE HYDROCHLORIDE

To ethereal extract, added one drop of alcoholic KOH. It was then heated, cooled and acidified with 0.5N hydrochloric acid. Violet colour developed upon addition of a drop of 1 % w/v $FeCl_3$ indicated presence of coumarins.

TEST FOR STEROLS

The powdered drug was first extracted with petroleum ether and evaporated to a residue. Then the residue was dissolved in chloroform and tested for sterols.

SALKOWSKI'S TEST

A few drops of concentrated sulphuric acid was added to the above solution, shaken well and set aside. The lower chloroform layer of the solution turned red in colour indicating the presence of sterols.

TEST FOR LIBBERMANN – BURCHARD'S

To the chloroform solution a few drops of acetic anhydride and 1 mL of concentrated sulphuric acid were added through the sides of the test tube and set aside for a while. At the junction of two layers a brown ring was formed. The upper layer turned green indicating the presence of sterols.

TEST FOR SAPONINS

FROTH TEST

0.1g of powder was vigorously shaken with 5ml of distilled water in a test tube for 30 seconds and was left undisturbed for 20 min, persistent froth indicated presence of saponins.

TEST FOR TANNINS

FERRIC CHLORIDE

Small quantity of the powdered drug was extracted with water. To the aqueous extract few drops of ferric chloride solution was added. Bluish black colour was produced indicating the presence of tannins.

GOLD BEATER'S SKIN TEST

Add 2 % hydrochloric acid to all small piece of g old beater's skin, rinses it with distilled water and place in the solution to be tested for five minutes. Then give wash of distilled water and transfer to a 1% ferrous sulphate solution. A brown or black colour on the skin indicates presence of tannin.

TEST FOR PHENOLIC COMPOUNDS

FERRIC CHLORIDE

A small quantity of the powdered drug was extracted with water. To the alcoholic extract few drops of ferric chloride solution was added. Bluish black colour was produced indicating the presence of tannins.

TEST FOR FOLIN COICALTEU REAGENT

To a drop of methanolic extract of a few drop of Folin Ciocalteu reagent was added, development of bluish green colour showed presence of phenol.

TEST FOR FLAVONOIDS

SHINODA'S TEST

Little of the powdered drug was heated with alcohol and filtered. To the test solution magnesium turnings and few drops of concentrated hydrochloric acid were added. Boiled for five minutes. Red colour was obtained indicating the presence of flavonoids.

ALKALI TEST

To the small quantity of test solution 10% aqueous sodium hydroxide solution was added. Yellow orange colour was produced indicating the presence of flavonoids.

LEAD ACETATE

To the test solution add a mixture of 10 % lead acetate in few drops added. It gives white precipitate.

TEST FOR ACID

To the small quantity of test solution, few drops of concentrated sulphuric acid were added. Yellow orange colour was obtained indicates the presence of flavonoids.

TEST FOR PROTEIN AND AMINO ACIDS

MILLON'S TEST

Small quantity of acidulous – alcoholic extract of the powdered drug was heated with Millon's reagent. White precipitate turned red on heating indicate the presence of proteins.

BIURET TEST

To one portion of acidulous – alcoholic extract of the powdered drug one ml of 10% sodium hydroxide solution and one drop of dilute copper sulphate solution were added. Violet colour was obtained indicating the presence of proteins.

NINHYDRIN TEST

To the test solution add Ninhydrin solution, boil, violet colour indicates presence of amino acid.

TEST FOR SULPHUR CONTAINING AMINO ACID

5 ml test solution is mixed with 2 ml 40 % sodium hydroxide and 2 drops of 10% lead acetate solution. Then boil the solution turned black or brownish due to PLS formation.

TEST FOR TERPENOIDS

Little of the powdered drug was extracted with chloroform and filtered. The filtrate was warmed gently with tin and thionyl chloride. Pink colour solution appeared which indicated the presence of terpenoids.

TEST FOR CAROTENOIDS (Carr-Price reaction)

Extract treated with concentrated sulphuric acid and with a chloroform solution of antimony trichloride. Deep blue colour appeared which indicated the presence of carotenoids.

TEST FOR VOLATILE OIL

Weighted quantity (250 gm) of fresh leaves were extracted and subjected to hydro distillation using volatile oil estimation apparatus.

TEST FOR FIXED OIL

A small amount of the powder was pressed in between in the filter paper and the paper was heated in an oven at 105^0 C for 10 minutes. A translucent greasy spot appeared indicating the papers.

TEST FOR GUM

The small quantity of extract was added with few drops of alcohol to form white precipitate which indicates the presence of gum.

TEST FOR MUCILAGE

Few ml of aqueous extract was prepared from the powdered crude drug was treated with ruthentium red. Red colour was produced indicating the presence of mucilage.

TEST FOR BETACYANINS

To 1 ml of plant extract, 1 mL of 2N NaOH was added and heated for 5 minutes at 100^0C. Formation of yellow colour indicated the presence of betacyanins.

TEST FOR ANTHOCYANIN

About 0.2g of plant extract was weighed in separate test tube, 1ml of 2N sodium hydroxide was added, and heated for 5 minutes. Observed for the formation of bluish green colour which indicates the presence of anthocyanin.

TEST FOR LEUCOANTHOCYANINS

To 1 ml of plant extract , 1 ml of isoamyl alcohol was added. Formation of red colour indicated the presence of leucoanthocyanins.

TEST FOR QUINONES

To 1 ml of plant extract, 1 mL of conc. H_2SO_4 was added. formation of red colour indicated the presence of quinones.

TEST FOR EMODINS

The dry extract was added to 25% ammonia solution. The formation of a cherry red colour solution indicated the presence of emodins.

TEST FOR COUMARINS

To 1 ml of plant extract, 3 ml of NH_4OH and 2mL of benzene was added. Formation of red colour indicated the presence of coumarin.

TEST FOR RESINS

The extracts were treated with acetone. A small amount of water was then added and shaken. Appearence of turbidity indicates the presence of resins.

TEST FOR PHLOBATANNINS

About 2 ml of aqueous extract was added to 2ml of 1% HCl and the mixture was boiled. Deposition of a red precipitate was an evidence for the presence of phlobatannins.

The results are presented in the **Table: 6**

THIN LAYER CHROMATOGRAPHY (TLC)

The hydroalcoholic extract was subjected to TLC by using different mobile phases as per Harborne J.B 1998, wagner 1996, to identify the R_f values. It was carried out using TLC plates. It was tested for its phenolic and flavonoid content by using mobile phases such as **Acetic acid : Chloroform (1:9), Ethyl acetate : Benzene (9:11)**, **Chloroform : Ethyl Acetate (60:40)**, **Ethyl Acetate : Formic Acid : Glacial Acetic Acid : Water (100:11:11:26)**. R_f value was measured in UV light at different nanometers.

The results are depicted in **Table: 7**

QUANTITATIVE ESTIMATION OF PHYTO CONSTITUENTS

DETERMINATION OF GALLIC ACID EQUIVALENT IN (HAECB) (Singleton *et al*., 1999)

Principle

Total phenolic content of the various concentrations of HAECB was determined by Folin-ciocalteu reagent method. The hydroxyl group (OH) of phenolic compounds reduce the phosphomolybdic acid to molybdenum blue in the presence of alkaline medium (present in Folin reagent). The blue coloured complex was then spectrophotometrically measured at 760nm.

Instrument

UV Visible spectrophotometer, Shimadzu (Model 1800).

Reagents required

- Folin-Ciocalteu Reagent (1N)
- Sodium carbonate solution (10%)
- Standard Gallic acid solution

Procedure

About 1 mL (1mg/ml and 0.5 mg/mL) of hydroalcoholic extract of *Commelina benghalensis L.* (Leaf) (HAECB), 0.5 mL of Folin-ciocalteu reagent (1N) were added and allowed to stand for 15 minutes. Then 1 mL of 10% sodium carbonate solution was added to the above solution. Finally the mixtures were made up to 10 mL with distilled water and allowed to stand for 30 minutes at room temperature and total phenolic content was determined spectrophotometrically at 760nm wavelength.

The calibration curve was generated by preparing gallic acid at different concentration (5, 10, 15, 20 and 25 µg/mL). The reaction mixture without sample was used as blank. Total phenolic content of HAECB extract is expressed in terms of mg of Gallic acid equivalent per gm of extract (mg GAE/g). The results are tabulated in **Table: 8** and the calibration graph was presented in **Fig: 14**

DETERMINATION OF TANNIC ACID EQUIVALENT IN (HAECB)

(RabiaNaz and AsghariBano, 2013)

Principle

Total tannin content of extract was determined by Folin Denis reagent method. Tannin like compounds reduces phosphotungstomolybdic acid in alkaline solution to produce a highly coloured blue solution. The intensity of which is proportional to the amount of tannins and it was estimated by spectrophotometer at 700 nm.

Instrument

UV Visible spectrophotometer, Shimadzu (Model 1800).

Reagents required

- Folin Denis Reagent
- Sodium carbonate solution (10%)
- Standard tannic acid solution.

Procedure

0.2 mL of (1 mg/mL) hydroalcoholic extract of *Commelina benghalensis L.* (Leaf) was made up to 1 mL with distilled water. Then add 0.5 mL of Folin Denis reagent and allowed to stand for 15 min, then 1 mL of sodium carbonate solution was added to the mixture and it was made up to 10 mL with distilled water. The mixture was allowed to stand for 30 min at room temperature and the tannin content was determined spectrophotometrically at 700nm.The calibration curve was

generated by preparing tannic acid at different concentration (5, 10, 15, 20 and 25µg/mL). The reaction mixture without sample was used as blank. The total tannin content in the leaf extract was expressed as milligrams of tannic acid equivalent per gm of extract. The results are tabulated in **Table: 9** and the calibration graph was presented in **Fig: 15**

DETERMINATION OF RUTIN (FLAVONOID) EQUIVALENT IN (HAECB)

(Zhishen *et al*., 1999)

Principle

Flavonoids present in the extract form, a charge transfer complex with several heavy metals to give a characteristic colour. In this reaction, the high electron positive nature of aluminium attracts the atomic nuclei of the aromatic rings in the flavonoids. Then it will react with potassium acetate in alkaline medium to form a pink coloured complex that is measured spectrophotometrically at 415 nm.

Instrument

UV Visible spectrophotometer, Shimadzu (Model 1800).

Reagents required

- 10% aluminium chloride
- 1M potassium acetate
- Standard rutin

Procedure

1mL of hydroalcoholic extract of *Commelina benghalensis L.* (Leaf), 0.1 mL of aluminium chloride solution, 0.1 mL of potassium acetate solution and 2.8 mL of ethanol were added and the final volume was then made up to 5 mL with distilled water. After 20 min the absorbance was measured at 415 nm.

A calibration curve was constructed by plotting absorbance reading of rutin at different concentrations (5, 10, 15, 20 and 25 µg/mL). The sample without aluminium chloride was used as a blank. The total flavonoid content in the extract was expressed as milligrams of rutin equivalent per gram of extract. The results are tabulated in **Table: 10** and the calibration graph is presented in **Fig: 16**

DETERMINATION OF CHLOROPHYLL "a", CHLOROPHYLL "b", TOTAL CHLOROPHYLL AND TOTAL CAROTENOID (Dhruve JJ *et al*., 2015)

Total chlorophyll, Chlorophyll "a", chlorophyll "b" and total carotenoids were estimated as described by Hiscox and Israelstam (1979). 50 mg fresh leaves were cut into small pieces and kept in 10ml dimethyl sulfoxide (DMSO) containing tube overnight. The extract was filtered through Whatman No.1 filter paper. Absorbance was measured in spectrophotometer at 645 nm and 663 nm for determination of total chlorophyll and absorbance was measured in spectrophotometer at 453nm for determination of total carotenoids.

$$\text{Chlorophyll "a" (mg/g fresh tissue)} = \frac{12.7 \times \text{O.D at 663 nm} - 2.69 \times \text{O.D at 645} \times 10}{1000 \times 0.05}$$

$$\text{Chlorophyll "b" (mg/g fresh tissue)} = \frac{22.9 \times \text{O.D at 645 nm} - 4.68 \times \text{O.D at 663} \times 10}{1000 \times 0.05}$$

$$\text{Total chlorophyll (mg/g fresh tissue)} = \frac{20.2 \times \text{O.D at 645 nm} + 8.02 \times \text{O.D at 663} \times 10}{1000 \times 0.05}$$

Total carotenoid = 0.216 × O.D 663 – 1.22 × O.D 645 – 0.354 × O.D at 663 + O.452 × O.D 453 (mg/g fresh tissue)

The results are depicted in **Table: 11 & 12**

LUTEIN

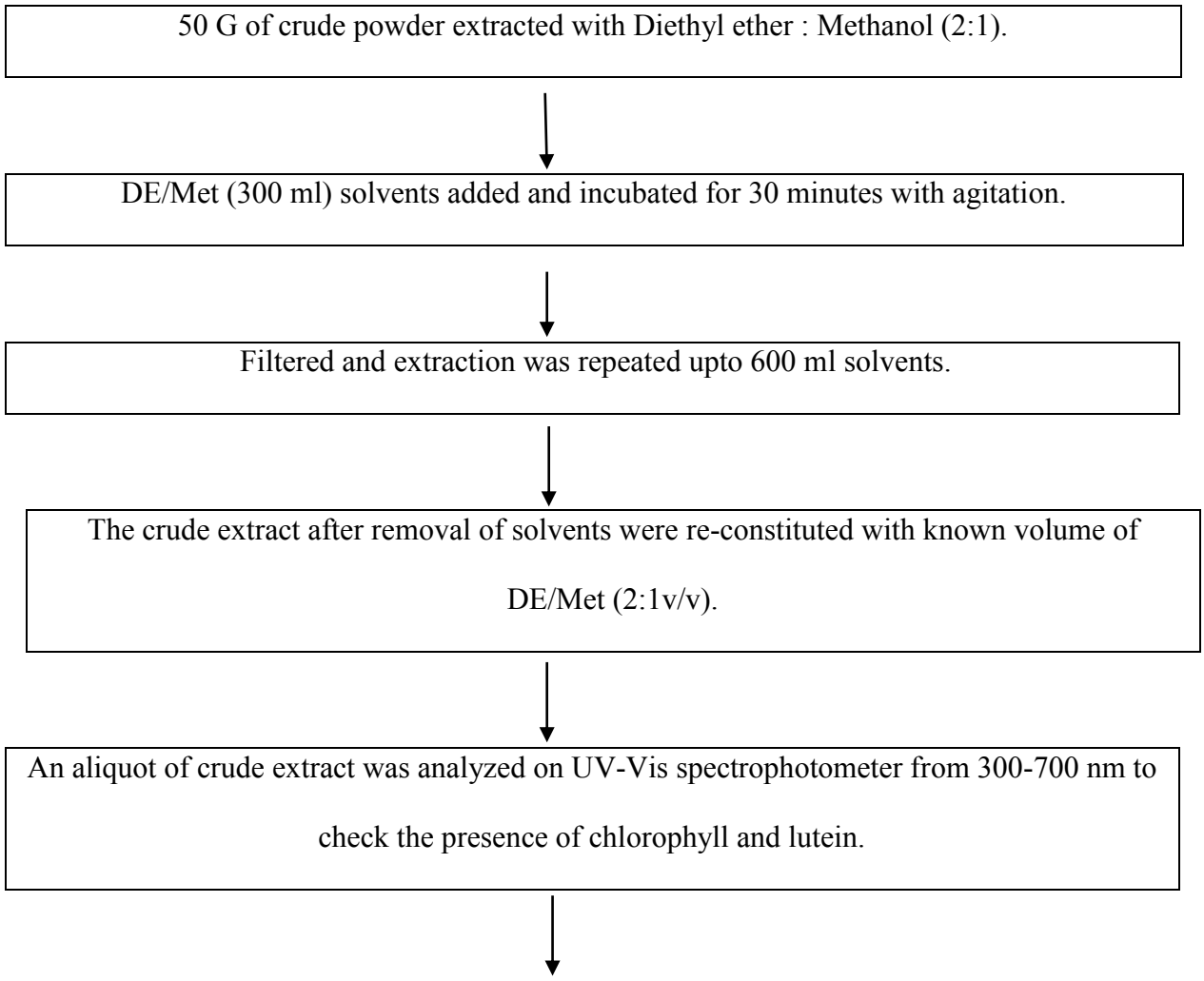
Carotenoids are fat soluble nutrients and categorized as either xanthophylls or carotenes according to their chemical structure. They are very important natural antioxidants that have wide application on human health benefits. The pigment properties of carotenoids have granted to some extensive application in the food and feed industries (Alves-Rodrigues & Shao, 2004). Carotenoids are naturally found in edible leaves, flowers and fruits. Currently attention is being drawn towards exploring plant sources for substances that provide nutritional and pharmaceutical advantages to humans. Green leafy vegetables (GLVs) are good sources of minerals and vitamins and also have health benefits. GLVs are rich sources of carotenoids especially lutein. Lutein a member of the xanthophylls family of carotenoids, decreases the risk for eye diseases such as Age-related Macular Degeneration (Seddon et al., 1994), protects the skin (Alves-Rodrigues & Shao, 2004), reduces cardiovascular problems, aging etc., and is being recommended for human, veterinary and poultry uses.

Lutein and zeaxanthin belong to the xanthophyll family of carotenoids and are the two major components of the macular pigment of the retina. Lutein and zeaxanthin differ from other carotenoids in that they each have two hydroxyl groups, one on each side of the molecule. Zeaxanthin is a stereoisomer of lutein, differing only in the location of a double bond in one of the hydroxyl groups. The hydroxyl groups appear to control the biological function of these two xanthophylls. The macula lutea or "yellow spot" in the retina is responsible for central vision and visual acuity. Lutein and zeaxanthin are the only carotenoids found in both the macula and lens of the human eye, and have dual functions in both tissues – to act as powerful antioxidants and to filter high-energy blue light. Lutein is found in high amounts in human serum. In the diet it is found in highest concentrations in dark green, leafy vegetables (spinach, kale, collard greens, and others), corn, and egg yolks. Zeaxanthin is the major carotenoid found in corn, orange peppers, oranges, and tangerines. In addition to playing pivotal roles in ocular health, lutein and zeaxanthin are important nutrients for the prevention of cardiovascular disease, stroke, and lung cancer. They may also be protective in skin conditions attributed to excessive ultraviolet (UV) light exposure.

Lutein and zeaxanthin are powerful antioxidants, and lutein is widely known as the primary nutrient for protecting ocular function. It has long been thought that carotenoid intake also reduces the risk of certain forms of cardiovascular disease, stroke, and cancer. Lutein and zeaxanthin may prevent cellular damage in these conditions by quenching singlet oxygen or neutralizing photosensitizers. Lutein and zeaxanthin inhibit lipid peroxidation, a likely factor in the etiology of both retinal and cardiovascular disease. The presence of adhesion molecules on endothelial cell surfaces is a marker of atherosclerosis pathogenesis. *In vitro* research has demonstrated lutein incubation with cultured endothelial cells effectively inhibits the expression

of these adhesion molecules. other research has found lutein and zeaxanthin can inhibit thickening of the walls of carotid arteries and LDL-induced migration of monocytes to human artery cell walls. These are potential mechanisms for lutein's protective effect in cardiovascular disease. In the case of skin health, lutein, zeaxanthin, and other carotenoids appear to be depleted in the skin under conditions of prolonged UV light exposure. Skin exposure to UV rays generates reactive oxygen species, inflammation in skin cells, and erythema. Intake of dietary antioxidants, including lutein and zeaxanthin, reduces this inflammatory response, as carotenoids are poor absorbers of UV light.

ISOLATION OF LUTEIN (Vatsala TM and Rekha R, 2013)



Crude extracts partitioned with saturated Nacl 1:1 (v/v) at (26 ± 2ºc) for 5 min followed by overnight saponification with methanolic KOH 10% at (26 ± 2ºc) .

↓

Saponified mixtures were transferred to separating funnel and equal volume of DE was added for further carotenoid separation from chlorophyll and incubated at (26 ± 2ºc) for 5 min, followed by addition of equal volume of distilled water to the saponified mixture.

↓

Shaken vigorously and allowed to stand for 15 – 20 min at 26 ± 2ºc.

↓

Bottom layer was removed

Top layer washed repeatedly with distilled water for complete removal of alkali.

↓

Top layer was analysed by spectrophotometer from 300 – 700 nm.

UV SPECTRAL STUDIES (Vatsala TM and Rekha R, 2013)

Lutein and Chlorophyll

Aliquot of crude extract was transferred to cuvette (quartz) and was scanned under UV range from 300 – 700 nm in the UV – Visible spectrophotometer to check the presence of chlorophyll and lutein. The results are tabulated in **Table: 13** and the spectrum was presented in **Figure: 17**

Lutein

The isolated compound lutein was dissolved in diethyl ether and transferred to cuvette (quartz) and was scanned under UV range from 300 – 700 nm in the UV – Visible spectrophotometer. The results are tabulated in **Table: 14** and the spectrum was presented in **Figure: 18**

NMR SPECTRAL STUDIES (Omayma A. Eldahshan *et al*., 2013)

^{1}H NMR

The Isolated compound was dissolved in Duterated chloroform ($CDCL_3$) and the NMR spectrum was obtained by using UXNMR Bruker Analytische Messtechnik Gmbh. Chemical shift values are tabulated in **Table: 15 & 16** and the spectrum was presented in **Figure: 19**.

^{13}C NMR

The Isolated compound was dissolved in Duterated chloroform ($CDCL_3$) and the NMR spectrum was obtained by using UXNMR Bruker Analytische Messtechnik Gmbh. Chemical shift values are tabulated in **Table: 17** and the spectrum was presented in **Figure: 20**.

GC-MS STUDIES

The isolated compound was subjected to GC-MS studies and the results are depicted in **Table: 18** and the spectrum was presented in **Figure: 21-21.24**.

PART C

PHARMACOLOGICAL STUDIES

SUN PROTECTION FACTOR (Manoj A. Suva; 2014)

Solar ultraviolet radiation (UVR) is divided into three categories UV-C (200-280 nm), UV-B (280-320) and UV-A (320- 400 nm). UV light has been classified by WHO as carcinogenic and produces several adverse effects including mutagenicity, immune depression of the skin, accelerated skin ageing and photo dermatoses (Nohynek and Schaefer, 2001). The most biologically damaging radiation UV-C has been filtered out by the ozone layer and it is mainly UV-B that is responsible for causing the adverse effects of the UV radiation (Kaur and Saraf, 2010; Mishra *et al.,* 2011). Application of sunscreen to the skin changes the way the body reacts to the sun rays (Mishra *et al.,* 2012). Sunscreens and sun blocks are chemicals that absorb or block UV rays and show a variety of immunosuppressive effects of sunlight. There are several agents available from both synthetic and natural sources with UV-filtering properties. Given their potential to produce considerable human local and systemic exposure, UV filters have to be safe (Nohynek *et al.,* 2010). Synthetic UV filters are known to have potential toxicity in humans and also showed ability to interfere only in selected pathways of multistage process of carcinogenesis (Chanchal and Saraf, 2009). In contrast, herbal botanical sunscreens are safe, widely accepted by consumers and also work in various ways, playing multiple roles in ameliorating the process of carcinogenesis (Guyer *et al.,* 2003).

SPF is a number given to sunscreen formulations to determine its effectiveness and it is also useful when applied about 2mg/cm . SPF numbers indicates the time period for the product up to which it protects the person while stay in the sun before burning. In order to protect the

skin against ultraviolet radiation, the formulation should have good SPF number and also the formulation should have wide range of absorbance between 290 and 400nm range. In the present research work, isolated lutein from *Commelina benghalensis L.* were subjected for SPF evaluation by ultraviolet spectroscopic method. SPF value for sunscreen above 2 is considered as having good sunscreen activity.

The effectiveness of a sunscreen is usually expressed by sun protection factor (SPF) which is the ratio of UV energy required to produce a minimal erythemal dose (MED) in protected skin to unprotected skin. A simple, rapid and reliable in vitro method of calculating the SPF is to screen the absorbance of the product between 290-320 nm at every 5 nm intervals. SPF can be calculated by applying the following formula known as Mansur equation (Kaur and Saraf, 2010; Mishra *et al.,* 2012):

$$\mathbf{SPF = CF \times \sum_{290}^{320} EE\ (\lambda)\ \times\ I\ (\lambda)\ \times\ Abs\ (\lambda)}$$

Where CF = correction factor (10), EE (λ) = erythmogenic effect of radiation with wavelength λ, Abs (λ) = Spectrophotometric absorbance values at wavelength λ. The values of EE x λ are constants

MATERIALS AND METHODS

MATERIALS

Ethanol, Isolated Lutein from *Commelina benghalensis L.* ,

SAMPLE PREPARATION

Lutein isolated from *Commelina benghalensis L.* 50 mg of isolated lutein is dissolved in 50ml of ethanol (1000 µg/ml). From this stock solution three different concentration (50, 100, 200 µg/ml) was prepared

METHODS

50, 100, 200 µg/ml of isolated lutein were subjected for SPF evaluation by ultraviolet spectroscopic method. Then spectrophotometer readings (Shimadzu 1800 UV-VIS Spectrophotometer) of these solutions were taken in wavelength ranging from 290 to 320 at 5nm interval and readings were noted down. SPF for isolated lutein can be calculated by applying the following formula known as Mansur equation (Kaur and Saraf, 2010; Mishra *et al.,* 2012):

$$\mathbf{SPF = CF \times \sum_{290}^{320} EE\ (\lambda)\ \times\ I\ (\lambda)\ \times\ Abs\ (\lambda)}$$

Where CF = correction factor (10), EE (λ) = erythmogenic effect of radiation with wavelength λ, Abs (λ) = Spectrophotometric absorbance values at wavelength λ. The values of EE x λ are constants.

The results of sun protection factor of lutein is depicted in **Table: 20, 21 & 22**.

ANTI-INFLAMMATORY ACTIVITY

Medicinal and culinary herbs are rich sources of anti-inflammatory compounds such as flavonoids. Pharmaceutical drugs are built upon a single molecule while herbal remedies contain different active ingredients. One of the wide spread complaint against modern medicines is its side effect that can be attributed to a single biochemical pathway that is triggered by the molecule of interest. On the contrary herbal medicines mediate multifaceted biochemical attack on inflammation due to the diversity and synergy of the anti-inflammatory compounds. Inflammation is a protective response by our immune system against organisms which cause cell injury (e.g., microbes, toxins) and deals with the consequences of such injury. It may be acute or chronic, depending up on the nature of stimulus and the effectiveness of initial reaction in eliminating the stimulus or the damaged tissues. The main components of inflammation are a vascular reaction and a cellular response, both are activated by mediators that are derived from plasma proteins and various cells. The outcome of acute inflammation is either elimination of the noxious stimulus followed by decline of the reaction and repair of the damaged tissue, or persistent injury resulting in chronic inflammation.

Inflammation is a complex biological response of vascular tissue to harmful stimuli, pathogens, irritants characterized by redness, warmth, swelling and pain. Prolonged inflammation leads to the rheumatoid arthritis, atherosclerosis, hey fever, ischemic heart diseases and inflammation is a common manifestation of infectious diseases like leprosy, tuberculosis, syphilis, asthma, inflammatory bowel syndrome, nephritis, vascularitis, celiac diseases, auto-immune diseases etc.

Anti-inflammatory drugs like NSAIDs used to reduce the swelling and pain of inflammation. But these agents carry the risk of gastro-intestinal toxicity, cardiovascular and other toxicity for prolonged use. For these reason, there is a need for ant-inflammatory drugs

having less severe side effects to use for chronic inflammatory disease as well. Therefore, in recent time, more interest is shown in alternative and natural drugs for treatment of various diseases, but there is a lack of proper scientific evidence.

Leukocytes, the key players of inflammatory response, can eliminate microbes and dead cells by phagocytosis, followed by their destruction in phagolysosomes. Destruction is caused by free radicals generated in activated leukocytes (neutrophils and monocytes) and lysosomal enzymes. Enzymes and reactive oxygen species may be released into the extracellular environment where it acts as mediators of inflammation. Such mediators are mainly arachidonic acid metabolites, generated through Cyclooxygenase and Lipoxygenase pathways. Most of the anti-inflammatory drugs are targeted on these pathways.

In a different approach, rather than blocking a particular mediator or its pathway, preventing the release of inflammatory mediators could be considered as a better option. The possibility of this approach is revealed in this research by studying the ability of the plant extract to prevent the lysosomal membrane destruction. An effective way to study this activity in vitro is to study the HRBC membrane stabilization activity of the plant extract. Lysosomal membrane and RBC membrane are similar in structure apart from the fact that luminal surface of the lysosomal membrane contains a glycoprotein coat which protects the membrane from digestion by lysosomal acid hydrolases. This method has been used in most preliminary anti-inflammatory screening procedures. Plants produce different bioactive compounds using secondary metabolic pathways in response to specific environmental stimuli such as herbivore-induced damage, pathogen attacks, or nutrient depravation. These secondary metabolites can be unique to specific species or genera and perform a host of general, protective roles including anti-inflammatory and antioxidant activities.

Commelina benghalensis L. commonly known as 'Benghal dayflower' or 'tropical spiderwort' is a perennial herb and its young leaves are eaten as vegetables. It is used as a folk medicine for the variety of ailments in the Indian subcontinent. It has antibacterial, sedative, anxiolytic, analgesic and anticancer properties and used against diuretic, febrifuge, inflammatory and leprosy problems. The phytochemical studies of *Commelina benghalensis L.* revealed the presence of flavonoids and phenolic compounds. The current study focuses on the evaluation of in vitro anti-inflammatory property and phytochemical nature of the leaf extracts of *Commelina benghalensis L.*

INVITRO ANTI-INFLAMMATORY ACTIVITY SCREENING BY MEMBRANE STABILIZATION STUDY (Sadique *et al.*,1989) (Oyedapo *et al.*, 2012)

Principle

The method of Sadique *et al.,* (1989) and modified by Oyedapo and Famurewa (1995) and Oyedapo *et al.,* (2012) was employed in the membrane stabilizing activity assay. When RBCs are subjected to heat and treatment with hyposaline they release haemoglobin which has a maximum absorbance at about 560 nm. The capacity of the extract to reduce hyposaline and heat induced lysis is basis of the assay.

Instrument

Shimadzu UV visible spectrophotometer, Model 1800

Materials required

70% Hydroalcoholic extract of *Commelina benghalensis L.*

0.2 M Sodium phosphate buffer (p^H 7.4)

0.36% w/v Hyposaline

10% v/v HRBC suspension in isosaline

Preparation of HRBC suspension in isosaline

The human erythrocytes suspension was used for the *in-vitro* membrane stabilization assay. Blood was collected from the healthy volunteers who had not consumed any NSAIDs for two weeks prior to the experiment. The blood was mixed with equal volume of Alsever's solution (2% dextrose, 8.0% sodium citrate, 0.5% citric acid, 0.42% sodium chloride) and centrifuged at 3000 rpm. The packed cells were washed with isosaline and a 10% v/v erythrocyte suspension in isosaline was prepared.

Procedure

The assay mixture consist of 2 ml of hyposaline and 1 ml of phosphate buffer and varying volumes (0.1 to 0.5 ml) of HAECB extract at different concentration (10, 20, 30, 40, 50 µg/ml) and 0.5 ml HRBC suspension in isosaline, then the final volume were made upto 4.5 ml with isosaline. The control was prepared as mentioned above without the test extract, while drug control was also prepared similarly but without HRBC suspension. The reaction mixture was incubated at 56º C for 30 min in a water bath, then the tube was cooled under running water. Then the absorbance of the released haemoglobin was measured at 560 nm. Diclofenac sodium was used as a reference standard. The percentage membrane stabilization activity of the compounds were determined by the formula

$$\% \text{ Membrane stabilization} = \frac{[A_{control} - (A_{test} - A_{product\ control})}{A_{control}} \times 100$$

Where

$A_{control}$ - Absorbance in control

A_{test} - Absorbance in test

$A_{product\ control}$ - Absorbance in product control

The results of membrane stabilization study is depicted in **Table: 23** and the calibration was presented at **Figure: 22**

RHEUMATOID ARTHRITIS

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by joint swelling, synovial inflammation and cartilage destruction and commonly lead to significant disability. According to WHO, 0.3-1% of the world population is affected from rheumatoid arthritis (RA) and among them females are three times more prone to the disease as compared to males. It caused by number of proinflammatory molecules released by macrophages including reactive oxygen species and ecosanoids such as prostaglandins, leukotrines and cytokines. The regulation of these mediators secreted by macrophages and other immune cells and modulation of arachidonic acid metabolism by inhibiting enzymes like cox and lox are the potential target for chronic inflammatory conditions. Eventhough various categories like immunosuppressants, NSAIDs, steroidal anti-inflammatory drugs are being used till now, the potential side effects give a limitation for their use. Now it is a growing concern all over for the development of new safe, potent, less toxic antiarthritic drug . Hence, there is a need to explore for more naturally available alternatives, so that their therapeutic values can be assessed and expanded.

Plants are one of the most important sources of medicines. India is known as the "Emporium of Medicinal plants" due to availability of several thousands of medicinal plants in the different bioclimatic zones anti-inflammatory diseases including rheumatoid arthritis are still one of the main health problems of the world's population. The use of natural remedies for the treatment of inflammatory and painful conditions has a long history, starting with Ayurvedic treatment, and extending to the European and other systems of traditional medicines. Plant drugs are known to play a vital role in management of inflammatory diseases.

INVITRO ANTIARTHRITIC ACTIVITY BY PROTEIN DENATURATION METHOD (Lavanya R *et al.*, 2010)

Rheumatoid arthritis is an autoimmune disorder. One among the cause for the disease is due to the denaturation of the protein. Antiarthritic activity was studied by inhibition of protein denaturation method.

Materials required

70% Hydroalcoholic extract of *Commelina benghalensis L.*

Diclofenac sodium

Bovine serum albumin (5% w/v aqueous solution)

Phosphate buffer (P^H 6.3)

Instrument

Shimadzu UV visible spectrophotometer, Model 1800

EXPERIMENTAL PROTOCOL

The following four solutions were prepared

1. **Test solution** (0.5 ml)

The test solution consists of 0.45 ml bovine serum albumin (5% w/v aqueous solution) and 0.05 ml of HAECB (10, 20, 30, 40, and 50 μg/ml concentration).

2. Test control solution (0.5 ml)

The test control solution consists of 0.45 ml bovine serum albumin and 0.05 ml distilled water.

3. Product control (0.5 ml)

The product control consists of 0.45 ml of distilled water and 0.05 ml of HAECB (10, 20, 30, 40, and 50 µg/ml concentration).

4. Standard solution

Standard solution consists of 0.45 ml of bovine serum albumin and 0.05 ml of Diclofenac sodium solution.

All the above test samples was adjusted to p^H 6.3 using a small amount of 1N hydrochloric acid. They were incubated at 37º C for 20 minutes and heated at 57º C for 3 minutes. Allow to cool and about 2.5 ml of phosphate buffer (p^H 6.3) was added to all the above solution. The absorbance was measured using UV spectrophotometer at 416 nm. The percentage inhibition of protein denaturation was calculated using the formula :

$$\text{Percentage inhibition} = 100 - \frac{\text{OD of test solution} - \text{OD of product control}}{\text{OD of test control}} \times 100$$

The control represents 100% protein denaturation. The results were compared with the standard drug diclofenac sodium treated sample.

The results of protein denaturation is depicted in **Table: 24** and the calibration was presented at **Figure: 23**

ANTIOXIDANT ACTIVITY

Oxygen derived free radicals like superoxide, hydrogen peroxide, hydroxyl and nitric oxide radicals are collectively termed as reactive oxygen species (ROS) and have been implicated in the pathogenesis of various diseases. (Halliwell, 1997; Lata and Ahuja, 2003). When generation of ROS overtakes the antioxidant defense of the cells, the free radicals start attacking the cell proteins, lipids and carbohydrates and this leads to a number of physiological disorders (Yu, 1992; Campbell and Abdulla, 1995; Cotran et al., 1999). The oxidative stress has also been implicated in the pathogenesis of diabetes, liver damage, nephrotoxicity, inflammation, cancer, cardiovascular disorders, neurological disorders, as well as in the process of aging. Many plants contain substantial amounts of antioxidants and can be utilized to scavenge the excess free radicals from human body.

Commelina benghalensis L. commonly known as 'Benghal dayflower' or 'tropical spiderwort' is a perennial herb and its young leaves are eaten as vegetables. It is used as a folk medicine for the variety of ailments in the Indian subcontinent. It has antibacterial, sedative, anxiolytic, analgesic and anticancer properties and used against diuretic, febrifuge, inflammatory and leprosy problems. Earlier phytochemical studies of *Commelina benghalensis L.* revealed the presence of lutein and its isomer zeaxanthin. The plant is also reported to contain alkaloids, wax, vitamin C and higher levels of vitamin A and β-carotene. The antioxidant activity DPPH radical scavenging activity of the hydro methanol extract of *C. benghalensis* has been reported by (Hasan *et al*., 2009)

Although some ethnobotanical and phytochemical information are available on these plants, their nutraceutical values have not yet been exposed. Therefore the antioxidant potential of these plants was investigated by employing different in vitro free radical scavenging assays.

INVITRO ANTIOXIDANT ACTIVITY

DETERMINATION OF SCAVENGING ACTIVITY AGAINST HYDROGEN PEROXIDE (Rana MG *et al.*, 1996)

Principle

The principle is based on the capacity of the extract to decompose the hydrogen peroxide to water. H_2O_2 in the presence of O^{2-} can generate highly reactive hydroxyl radicals via the metal, the scavenging of H_2O_2 in cells is critical to avoid oxidative damage. Thus, the scavenging of hydrogen peroxide is an important antioxidant defence mechanism.

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH + OH^-$$

The decomposition of hydrogen peroxide to water involves the transfer of electrons as in the equation.

$$H_2O_2 + 2H^+ + 2e^- \longrightarrow 2H_2O$$

Reagents

- 6% hydrogen peroxide diluted with water in the ratio of 1:10
- 0.1 M Phosphate buffer (pH 7.4)

Procedure

To 1 mL of test solutions of different concentrations, 3.8 mL of 0.1 M phosphate buffer solution (pH 7.4) and then 0.2 mL of hydrogen peroxide solution were added. The absorbance of the reaction mixture was measured at 230 nm after 10 min. The reaction mixture without sample was used as blank. Sample blank was also prepared without reagents. Ascorbic acid was used as standard. The percentage inhibition of hydrogen peroxide was calculated using the formula,

% inhibition = [(Control–Test) / Control] × 100

The concentration of the sample required for 50 % reduction in absorbance (IC_{50}) was calculated using linear regression analysis.

The results are tabulated in **Table: 25** and the calibration graph was presented in **Figure: 24&25.**

DETERMINATION OF REDUCING POWER ASSAY (Navnath *et al.*,2010)

Principle

Reducing power assay is a spectrophotometric method and is based on the principle that increases absorbance of the reaction mixture indicates the increase in the reducing power of the sample. Antioxidant activity may be due to a variety of mechanism viz., the prevention of chain initiation, the binding of transition metal ion catalysts, decomposition of peroxides, the reducing capacity and free radical scavenging. The assay is based on the reduction of ferric in potassium ferricyanide to ferrous to form potassium ferrocyanide by the sample and the subsequent formation of Prussian blue colour with ferric chloride. The absorbance of the blue complex is measured at 700 nm.

Potassium ferricyanide+ Ferric chloride

↓ **Antioxidant**

Potassium ferrocyanide + Ferrous chloride

Instrument

UV Visible spectrophotometer, Shimadzu (Model 1800).

Materials required

- Ascorbic acid
- 1 % w/v Potassium ferricyanide
- 10 % w/v Trichloro acetic acid
- 0.2 M, Phosphate buffer (pH 6.6)
- 0.1% w/v Ferric chloride

Procedure

The reducing power ability of plant extracts was screened by assessing the ability of the test extract to reduce $FeCl_3$ solution as mentioned by Oyaizu *et al.,* (1986). 0.1 to 0.5 mL of plant extract solution (1 mg/mL) was mixed with 0.75 mL of phosphate buffer and 0.75 mL of 1 % potassium ferricyanide [$K_3Fe(CN_6)$] and incubated at 50^oC for 20min. About 0.75 mL of 10 % trichloro acetic acid was added to the mixture and allowed to stand for 10min. The whole mixture was then centrifuged at 3000 rpm for 10min. Finally 1.5 mL of the supernatant was

removed and mixed with 1.5 mL of distilled water and 0.1mL of 0.1 % ferric chloride solution and the absorbance was measured at 700 nm in UV-Visible Spectrophotometer. Ascorbic acid was used as standard and phosphate buffer was used as blank solution. The results are tabulated in **Table: 26** and the calibration graph was presented in **Figure: 26&27.**

DETERMINATION OF TOTAL ANTIOXIDANT ACTIVITY (Prieto *et al*., 1999)

Principle

The total antioxidant activity of the extract was evaluated by phosphomolybdenum method. The assay is based on the reduction of Mo (VI) to Mo (V) by the sample and by the subsequent formation of green phosphate Mo (V) complex at acidic pH which has a maximum absorption at 695 nm. This method is routinely used to determine total antioxidant activity of samples.

$Mo^{6+} \rightarrow Mo^{5+}$

Instrument

UV Visible spectrophotometer, Shimadzu (Model) 1800.

Reagents

- 0.6M sulphuric acid
- 28mM sodium phosphate
- 4mM ammonium molybdate

Procedure

An aliquot of 0.3 mL of different concentrations of sample was treated with 2.7 mL of the reagent (H_2SO4, sodium phosphate and ammonium molybdate). In case of blank, 0.3 mL of methanol was used in place of sample. The tubes were incubated in a boiling water bath at 95°C for 90 min. The samples were cooled to room temperature, the absorbance of the aqueous solution of each concentration was measured at 695 nm against blank. The standard vitamin C was treated in a similar manner. The antioxidant activity was expressed as equivalents of Vitamin C (µg/mL). The results are tabulated in **Table: 27** and the calibration graph was presented in **Fig: 28&29.**

CHAPTER-5

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

MACROSCOPICAL STUDIES

Fresh leaves of *commelinabenghalensis.L* was subjected to macroscopical studies and the results are presented in **Table:1**.

Table:1 Macroscopical studies of C*ommelinabenghalensis.L*(Leaf).

S.NO	PARAMETERS	OBSERVATION
1	Colour/surface	
	Outer Inner	Dull green Pale green
2	Odour	No characteristic
3	Taste	Mucilaginous
4	Type	Simple
5	Shape	Ovate
6	Phyllotaxy	Alternate
7	Apex	Acute
8	Base	Unequal
9	Stipules	Present
10	Margin	Entire
11	Venation	Parallel
12	Surface	Sheathing
13	Length	2.8-7.6 cm
14	Width	1.9-3.1 cm
15	Petiole length	0.8-2.3 cm

Macroscopical studies of *Commelinabenghalensis.L*revealed the outer surface showed dull green and the inner surface showed pale green with mucilaginous taste and odourless. The leaves are simple, ovate shape with acute apex, unequal base with stipules.The leaves are entire margin, parallel venation with alternate arrangement, surface sheathing. The leaves are of 2.8-7.6 cm in length and 1.9-3.1 cm width and its petiole length 0.8-2.3 cm.

Figure:1 Habitat of *Commelina Benghalensis L.*

Figure: 2 Macroscopy of *Commelina benghalensis L.* (Leaf) (Dorsal view)

Figure: 3 Macroscopy of *Commelina benghalensis L.* (Leaf) (Ventral view)

Figure: 4 Macroscopy of Flowers of *Commelina benghalensis L.*

Figure: 5 Stipules of *Commelina benghalensis L.*

MICROSCOPICAL STUDIES

Figure: 6 Transverse Section of Leaves

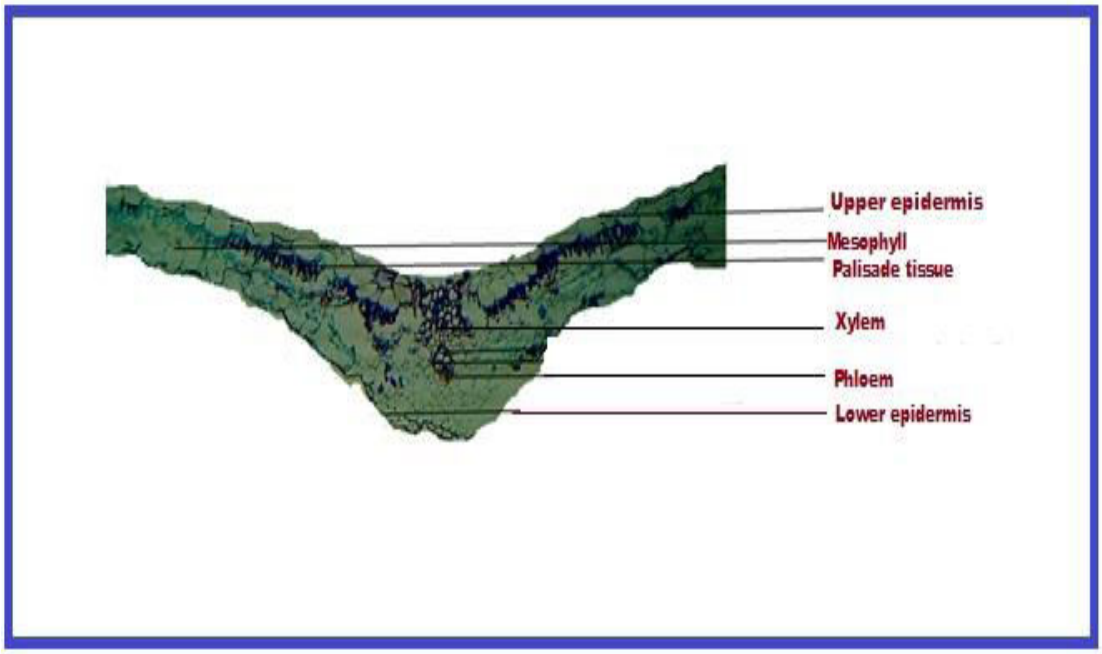


Figure: 7 Transverse Section of Midrib Enlarged

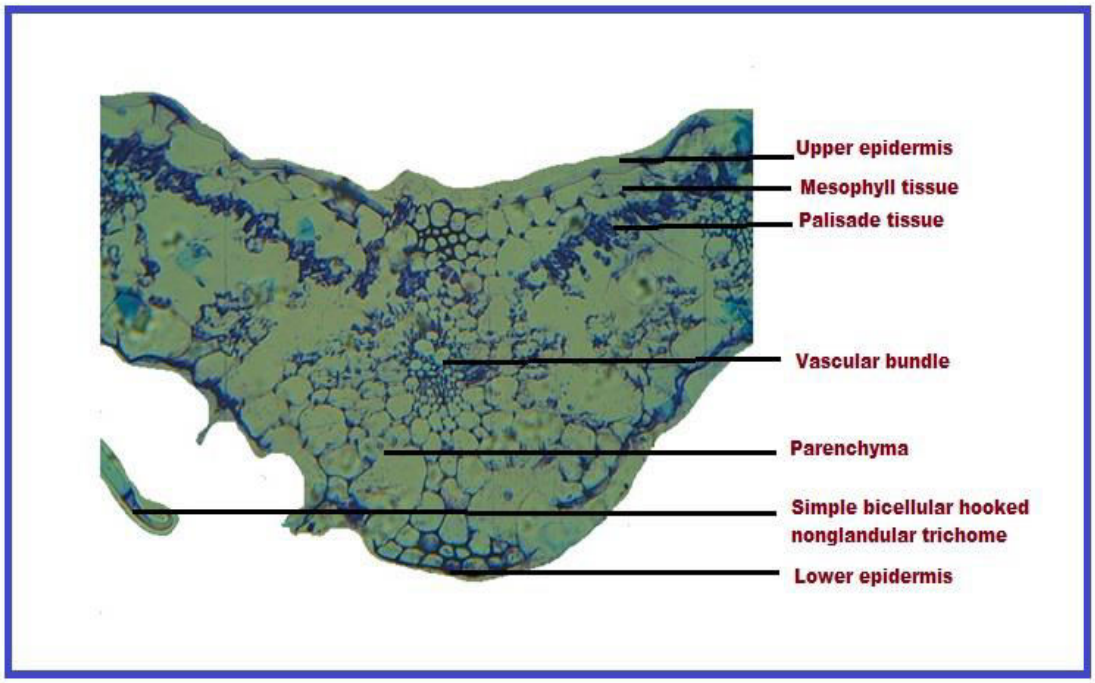


Figure: 8 Transverse Section of Leaf Sheath – Meristem Region

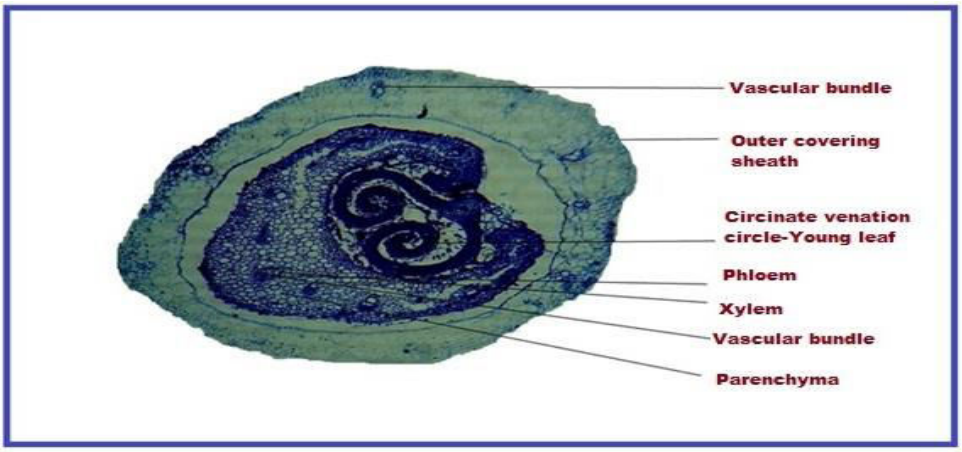


Figure: 9 Transverse Section of Shoot Tip – Meristem Region

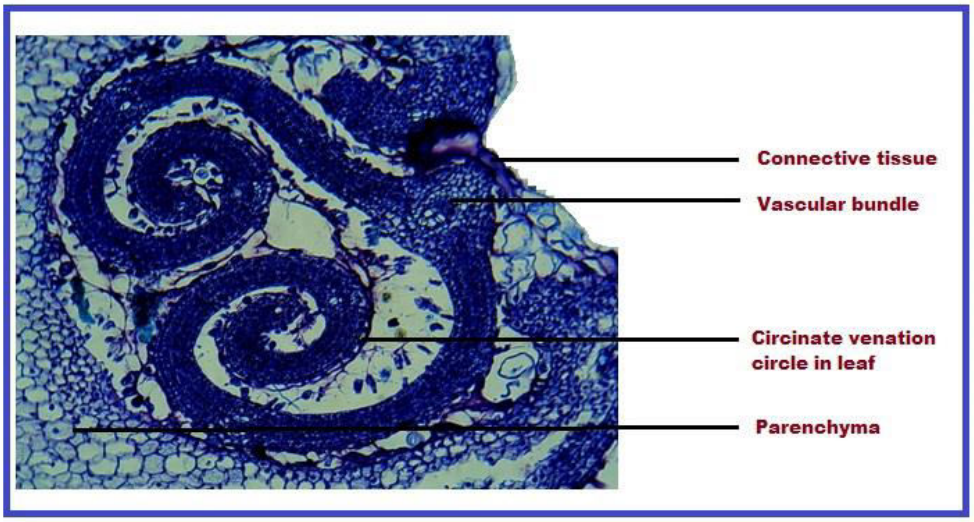


Figure: 10 Transverse Section of Lamina Enlarged

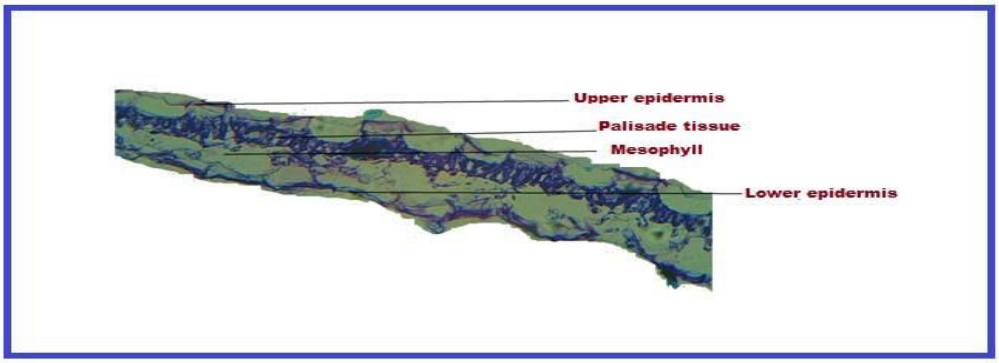


Figure: 11 Hexacytic stomata of *Commelina benghalensis L.*

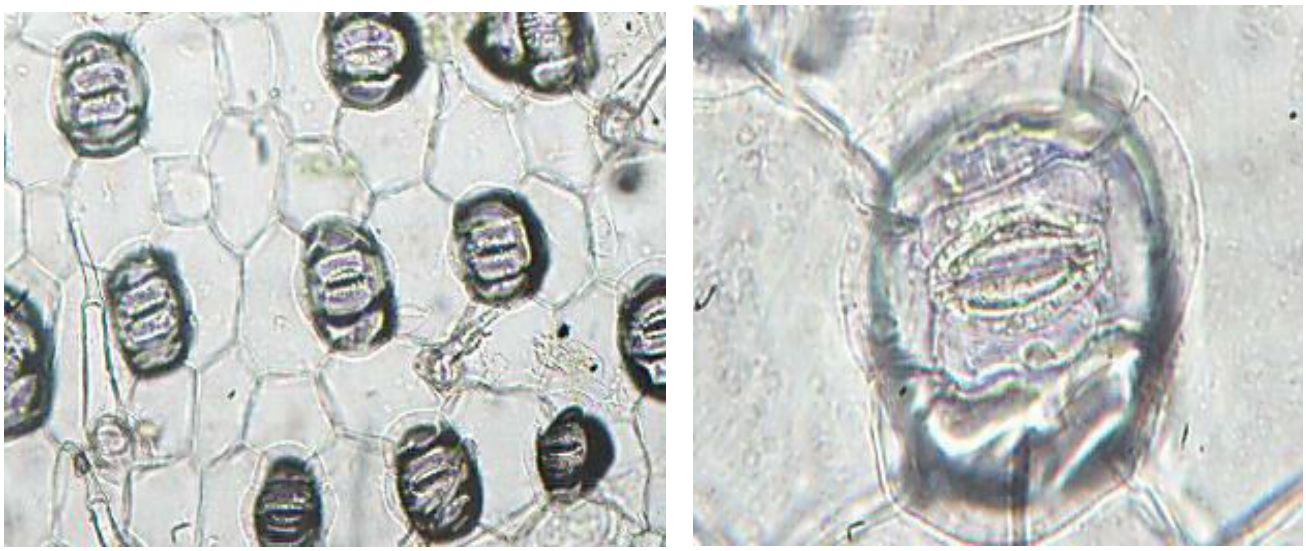
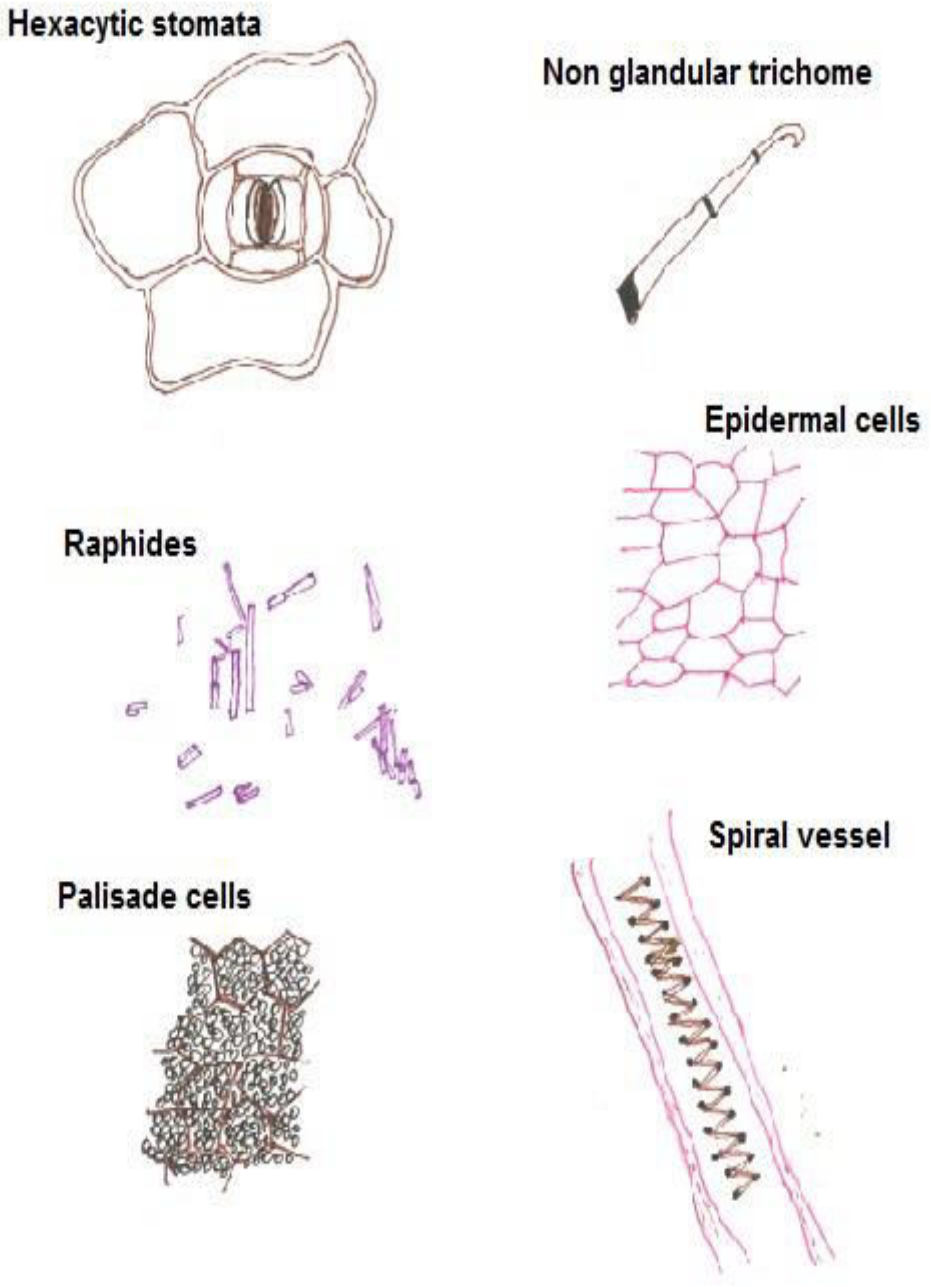


Figure: 12 Non Glandular Trichomes of *Commelina benghalensis L.*

Figure: 13 Powder microscopy of *Commelina benghalensis L.*



MICROSCOPICAL STUDIES

TRANSVERSE SECTION OF LEAF

Epidermis

Transverse section of *Commelinabenghalensis L.*leaves consists of an upper epidermis and lower epidermis. The epidermal cells are polygonal in shape.

Stomata

Amphistomatic types of stomata were present on both surfaces and abaxial side of the epidermis is more frequently. Hexacytic type of stomata were present.

Trichomes

The presence of simple bicellular hooked non glandular trichomes on both surface of leaves.

Ground Tissue

The ground tissue system of leaf is known as mesophyll tissue. The presences of mesophyll tissue have lot of air spaces. Stone cells of astrosclereids and osteosclereids were present in the mesophyll tissue. Crystals and starch grains are present in the mesophyll tissue.

Vascular Bundles

The vascular bundles are collateral and closed. Kidney shaped, Xylem vessels and trachids are also present in the vascular bundle.

TRANSVERSE SECTION OF MIDRIB ENLARGED

Epidermis

Transverse section of *Commelinabenghalensis L.*leaves consists of an upper epidermis and lower epidermis. The epidermal cells are polygonal in shape.

Trichomes

The presence of simple bicellular hooked non glandular trichomes on both surface of leaves.

Ground Tissue

The ground tissue system of leaf is known as mesophyll tissue. The presences of mesophyll tissue have lot of air spaces. Stone cells of astrosclereids and osteosclereids were present in the mesophyll tissue. Crystals and starch grains are present in the mesophyll tissue.

Vascular Bundles

The vascular bundles are collateral and closed. Kidney shaped, Xylem vessels and trachids are also present in the vascular bundle.

TRANSVERSE SECTION OF LEAF SHEATH – MERISTEM REGION

Transverse section of *Commelinabenghalensis L.* leaf sheath shows outer covering sheath. Circinate venation circle of young leaves present in meristem region.

Vascular Bundles

Vascular bundles are collateral and closed. Vascular bundles consist of xylem and phloem. Outer covering sheath also consists of vascular bundles.

TRANSVERSE SECTION OF SHOOT TIP – MERISTEM REGION

Transverse section of *Commelinabenghalensis L.* leaf shoot tip shows Circinate venation circle of young leaves. Vascular bundles are collateral and closed. Vascular bundle consists of xylem and phloem.

TRANSVERSE SECTION OF LAMINA & LAMINA ENLARGED

Transverse section of *Commelinabenghalensis L.* lamina consists of upper epidermis and lower epidermis. Palisade tissue and mesophyll tissue presented in the lamina.

QUANTITATIVE MICROSCOPY OF *CommelinaBenghalensis L.* LEAVES

Fresh leaves of *Commelinabenghalensis L.* was subjected to microscopical studies includes in stomatal number, stomatal index were determined and the results are showed in **Table: 2**

Table: 2 Quantitative Microscopy of *Commelinabenghalensis L.*Leaves

S.NO	Parameters	Minimum (per mm^2)	Average (per mm^2)	Maximum (per mm^2)
1	Stomatal Number			
	Lower Epidermis	39	48	42.6
	Upper Epidermis	15	20	17.8
2	Stomatal Index			
	Lower Epidermis	30.7	34.5	32.0
	Upper Epidermis	13.8	20.8	17.32

Determination of Physio – Chemical constants of *Commelinabenghalensis L.* (Leaf)

The powder was subjected to physiochemical parameters such as foreign organic matter, loss on drying, ash values and extractive values with different solvents in increasing order of polarity, volatile oil, and total solids and the results are showed in Table: 3

Table:3 Determination of Physio – Chemical Constants of *Commelinabenghalensis L.* (Leaf)

S.NO	PHYSIO – CHEMICAL CONSTANTS	REPORTS
1	Foreign matter	Nil
2	Loss on drying	0.78 ± 0.11% w/w
3	Total solids	99.22 ± 0.11 % w/w
4	Bitterness value	Nil
5	Volatile oil content	Nil
6	Petroleum ether extractive	0.6 ± 0.12% w/w
7	Ethyl acetate extractive	1.6 ± 0.12% w/w
8	Chloroform extractive	1.7 ± 0.07% w/w
9	Methanol extractive	4.4 ± 1.29% w/w
10	Aqueous extractive	12.13 ± 1.30% w/w
11	Total ash	16 % w/w

12	Water soluble ash	10.5 % w/w
13	Acid insoluble ash	1.17 % w/w

Physio – chemical constants of *Commelinabenghalensis L.* (Leaf – crude powder) was found to be, Loss on drying (0.78 ± 0.11% w/w), Total Solids (99.22 ± 0.11 % w/w). The powder did not possess any foreign matter, bitter principle and volatile oil content. The powder also exhibits Petroleum ether extractive value (0.6 ± 0.12% w/w), Ethyl acetate extractive values (1.6 ± 0.12% w/w), Chloroform extractive value (1.7 ± 0.07% w/w), Methanol extractive values (4.4 ± 1.29% w/w), Aqueous extractive values (12.13 ± 1.30% w/w), Total ash value (16 % w/w), Water soluble ash value (10.5 % w/w), Acid insoluble ash value (1.17 % w/w)

Behavioural characters of the *Commelinabenghalensis L.* (Leaf – crude powder) with different reagents.

Crude powder of leaf was treated with various reagent and the results are showed in Table: 4

Table:4 Behavioural characters of the *Commelinabenghalensis L.* (Leaf – crude powder) with different reagents.

Crude powder of *Commelinabenghalensis L.* (Leaf)	**Visible light**	**UV Light (254 nm)**	**UV Light (365 nm)**
Powder + water	Black	Brown	Did not show any characteristic change
Power + Con.HCL	Black	Brown	Did not show any characteristic change

Powder + Con.H_2SO_4	Black	Dull brown	Did not show any characteristic change
Powder + Con.HNO_3	Yellow	Green	Did not show any characteristic change
Powder + CH_3COOH	Black	Dull green	Did not show any characteristic change
Powder + Con.HCL + Water	Black	Dull green	Did not show any characteristic change
Powder + Con.H_2SO_4 + Water	Black	Dull green	Did not show any characteristic change
Powder + Con.HNO_3 + Water	Yellow	Dull green	Did not show any characteristic change
Powder + CH_3COOH + Water	Yellowish brown	Dull green	Did not show any characteristic change
Powder + Aqueous NaOH	Black	Dull green	Did not show any characteristic change
Powder + Aqueous $FeCl_3$	Black	Dull green	Did not show any characteristic change

Crude powder when treated with Water, Con.HCl, Con H_2SO_4, CH_3COOH, Con HCl + Water, Con H_2SO_4 + Water and Aqueous NaOH, Aqueous $Fecl_3$ showed **black colour** in visible light. Powder treated with Con.HNO_3 and Con.HNO_3 + Water showed **yellow colour** in visible light. Powder treated with CH_3COOH + Water showed yellowish **brown colour** in visible light. Powder when treated with Water and Con.HCl showed **brown colour** in UV 254 nm. Powder treated with Con.H_2SO_4 showed **dull brown** colour in UV 254 nm. Powder treated with

Con.HNO_3 showed **green colour** in UV 254 nm. Powder when treated with CH_3COOOH, Con.HCl + Water, Con.H_2SO_4 + Water, Con.HNO_3 + Water, CH_3COOH + Water, Aqueous NaOH, Aqueous $Fecl_3$ showed **dull green** colour in UV 254 nm. The powder did not show any characteristic colour change with the reagent under UV 366 nm.

Determination of physical parameters of (HAECB)

Hydro alcoholic extract of *Commelinabenghalensis L.* was subjected to physical parameter such as weight/ml, refractive index, consistency, colour and the results are displayed in **Table: 5**

Table:5 Determination of physical parameters of (HAECB)

S.NO	PARAMETERS	REPORTS
1	Refractive Index	1.342 ± 0.002
2	Weight/ml	0.882 ± 0.010
3	Consistency	Semi solid
4	Colour	Dark Brown

The physical parameters of hydro alcoholic extracts of *Commelinabenghalensis L.* (Leaf) such as refractive index, weight per ml, consistency and colour was determined. It was found to be refractive index (1.342 ± 0.002), weight per ml (0.882 ± 0.010), dark brown in colour with semisolid consistency.

Preliminary phytochemical screening of Aqueous and Hydro – Alcoholic extract of *Commelinabenghalensis L.* (Leaf)

Hydroalcoholic extract of *Commelinabenghalensis L.* (Leaf) was subjected to qualitative chemical analysis. The various chemical tests were performed on this extract and aqueous extract for the identification of phytochemicals, secondary metabolites and the results are displayed in **Table: 6**

Table: 6 Preliminary phytochemical screening of Aqueous and Hydro – Alcoholic extract of *Commelinabenghalensis L.* (Leaf)

S.NO	Test	Hydroalcoholic extract of *Commelinabenghalensis L.* (Leaf)	Aqueous extract of *Commelinabenghalensis L.* (Leaf)
1	**Alkaloids**		
	Mayer' Test	Positive	Positive
	Dragendorff's Reagent	Negative	Negative
	Hager's Reagent	Positive	Positive
	Wagner's Reagent	Positive	Positive
2	**Carbohydrates**		
	Benedict's Test	Positive	Positive
	Fehling's Test	Positive	Positive
	Molisch's Test	Positive	Negative
3	**Anthraquinone Glycoside**		
	Borntrager's Test	Negative	Negative
	Modified Borntrager's Test	Negative	Negative

4	**Cardiac Glycosides**		
	Keller killiani Test	Negative	Negative
	Legal Test	Negative	Negative
5	**Sterols**		
	Salkowski's Test	Positive	Positive
	Libbermann-Burchard's Test	Negative	Negative
6	**Saponins**	Positive	Positive
7	**Tannins and Phenolic compounds**		
	Folinciocalteu's phenol Reagent	Positive	Positive
	$Fecl_3$ Test	Positive	Positive
8	**Flavonoids**		
	Shinoda Test	Positive	Positive
	Lead Acetate Test	Positive	Positive
	Acid Test	Positive	Positive
	Alkali Test	Positive	Positive
9	**Protein and Free Amino Acids**		
	Biuret Test	Positive	Negative
	Ninhydrin Test	Positive	Positive
	Sulphur containing Amino Acid	Positive	Positive
10	Mucilage	Positive	Positive
11	Quinone	Positive	Positive
12	Phlobatannins	Positive	Positive
13	Carotenoids	Positive	Positive

14	Terpenoids	Positive	Negative
15	Betacyanins	Positive	Negative
16	Emodin	Negative	Positive
17	Fixed oil	Negative	Negative
18	Gum	Negative	Negative
19	Anthocyanins	Negative	Negative
20	Lecoanthocyanins	Negative	Negative
21	Resins	Negative	Negative
22	Volatile oil	Negative	Negative

The phytochemical screening of the hydroalcoholic extract (70%) of *Commelinabenghalensis L.* (Leaf) powder revealed the presence of alkaloids, carbohydrates, sterols, saponins, tannins and phenolic compound, flavonoids, protein and free aminoacid, terpenoids, mucilage, betacyanin, quinone, phlobatannins, carotenoids. It shows the absence of anthraquinone glycosides, cardiac glycoside, fixed oil, anthocyanin, lecoanthocyanin, volatile oil,emodin, gum, resins,. The aqueous extract of *Commelinabenghalensis L.* (Leaf) powder revealed the presence of alkaloids, carbohydrates, sterols, saponins, tannins and phenolic compound, flavonoids, protein and free aminoacid, mucilage, emodin, quinone, phlobatannins, carotenoids. It shows the absence of anthraquinone glycosides, cardiac glycoside, terpenoids, fixed oil, betacyanin, gum, anthocyanin, lecoanthocyanin, resins, volatile oil.

THIN LAYER CHROMATOGRAPHY PROFILE

Hydro alcoholic extract of *Commelinabenghalensis L.* was subjected to TLC by using different mobile phases to identify the R_f value and the results are presented in Table: 7

Table:7 TLC PROFILE OF HAECB

S.NO	Mobile Phase	R_f Value		Detection	Spot colour	Report
		Standard	**HAECB**			
1	Acetic acid : Chloroform (1:9)	**Gallic acid** 0.25	**HAECB** 0.25 0.90	Folin Reagent	Blue colour	May indicate the presence of **Phenolic** compound
2	Ethyl Acetate : Benzene (9:11)	**Gallic acid** 0.72	**HAECB** 0.73 0.96	Folin Reagent	Blue colour	May indicate the presence of **Phenolic** compound
3	Chloroform : Ethyl Acetate (60:40)	**Rutin** 0.46	**HAECB** 0.71	Visible light	Yellow	May indicate the presence of **Flavonoids, Flavones, Flavonols**
4	Ethyl Acetate : Formic Acid : Glacial Acetic Acid : Water (100 : 11 : 11: 26)	**Rutin** 0.42	**HAECB** 0.45 0.93 0.97	Visible light	Orange Yellowish green Yellow	May indicate the presence of **Flavonoids, Flavones, Flavonols**

Thinlayer chromatography of the hydroalcoholic extract of *Commelinabenghalensis L.* (Leaf) showed the R_f value 0.25(Gallic acid standard) 0.25, 0.90(sample) may indicate the presence of phenolic compounds the solvent system used in **Acetic acid : Chloroform (1:9)**. R_f value 0.72 (Gallic acid standard), 0.73,0.90 (sample) may indicate the presence of phenolic compounds the solvent system used in **Ethyl acetate : Benzene (9:11)**. R_f value 0.46 (Rutin standard) 0.71, may indicate the presence of flavonoids the solvent system used in **Chloroform : Ethyl Acetate (60:40)**. R_f value 0.42 (Rutin standard), 0.45,0.93,0.97 may indicate the presence of flavonoids, flavones, flavonols the solvent system used in **Ethyl Acetate : Formic Acid : Glacial Acetic Acid : Water (100:11:11:26)**.

QUANTITATIVE ANALYSIS

Quantitative analysis includes estimation of total gallic acid, total tannin, total flavonoid contents in terms of total gallic acid equivalent, total tannic acid equivalent, total flavonoids equivalent (rutin). In addition total carotenoid and total chlorophyll content were determined for the fresh leaf and were dispalyed in seperateTables.

DETERMINATION OF GALLIC ACID EQUIVALENT IN (HAECB)

Hydroalcoholic extract of *Commelinabenghalensis L.* was found tocontain **112 mg/g** of GAE and the standard curve of gallic acid and its results were displayed in **Table: 8 and Figure: 14**.

Figure: 14 Standard Curve of Gallic Acid

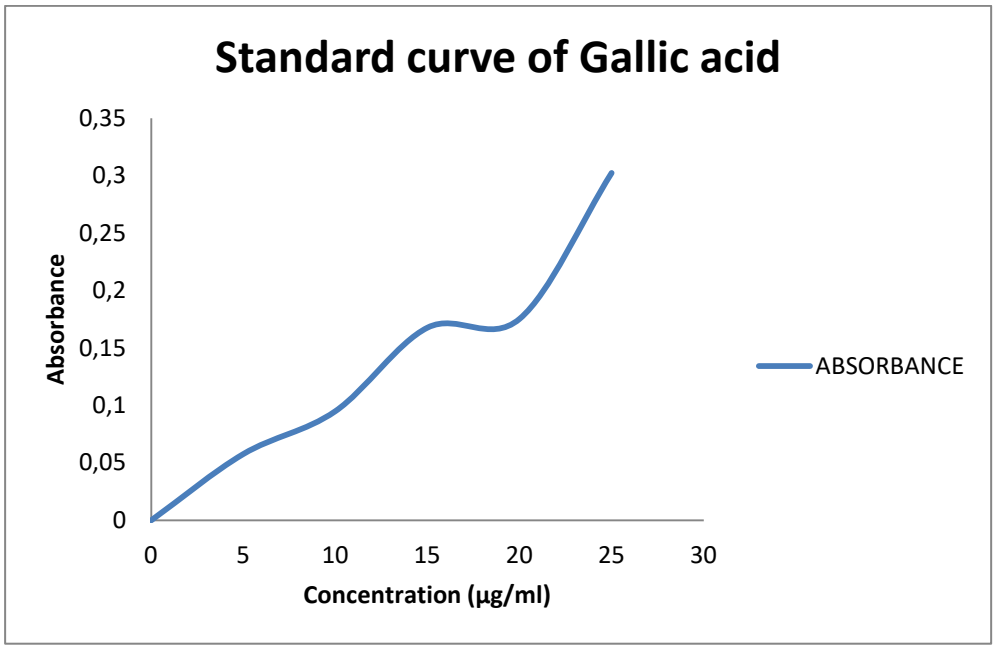


Table: 8 Determination of Gallic Acid Equivalent in (HAECB)

Sl.no	Concentration		Absorbance		Amount of Gallic Acid equivalent (GAE) in (HAECB) (µg/ml)
	Gallic Acid (µg/ml)	HAECB (µg/ml)	Gallic Acid *Mean ± SEM	HAECB *Mean ± SEM	
1	5	10	0.0577 ± 0.008	0.0067 ± 0.0006	1.06
2	10	20	0.095 ± 0.00110	0.0163 ± 0.0008	1.94
3	15	30	0.1677 ±0.0008	0.0357 ± 0.0008	3.7
4	20		0.1753 ± 0.0008		
5	25		0.3023 ± 0.0008		
				GAE	112 mg/g

*mean ± SEM

Hydroalcoholic extract of *Commelinabenghalensis L.*was found tocontain**112 mg/g** of GAE and the standard curve ofgallic acid and its results were displayed in **Table: 8 and Figure: 14**.

DETERMINATION OF TANNIC ACID EQUIVALLENT IN HAECB

Hydroalcoholic extract of *Commelinabenghalensis L.* was found tocontain **198 mg/g** of TAE and the standard curve of tannic acid and its results were displayed in **Table: 9 and Figure: 15**.

Figure: 15 Standard Curve of Tannic Acid

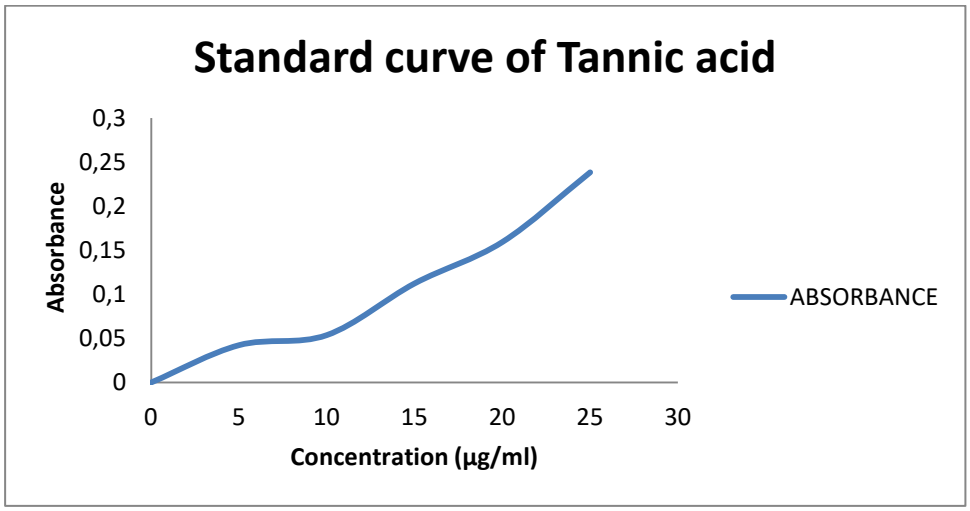


Table: 9 Determination of Tannic Acid Equivallent in HAECB

Sl. No	Concentration		Absorbance		Amount of Tannic Acid equivalent (TAE) in (HAECB) (µg/ml)
	Tannic Acid (µg/ml)	HAECB (µg/ml)	Tannic Acid *Mean ± SEM	HAECB *Mean ± SEM	
1	5	10	0.0423 ± 0.0008	0.0033 ± 0.0003	1.81
2	10	20	0.0537 ± 0.0008	0.012 ± 0.0005	2.78
3	15	30	0.1123 ± 0.0008	0.0527 ± 0.0008	7.3
4	20		0.1593 ± 0.0008		
5	25		0.2383 ± 0.0008		
				TAE	198 mg/g

*mean ± SEM

Hydroalcoholic extract of *Commelinabenghalensis L.* was found tocontain **198 mg/g** of TAE and the standard curve of tannic acid and its results were displayed in **Table: 9 and Figure: 15**.

DETERMINATION OF RUTIN EQUIVALLENT IN HAECB

Hydroalcoholic extract of *Commelinabenghalensis L.* was found tocontain **989 mg/g** of RE and the standard curve of rutin and its results were displayed in **Table: 10 and Figure: 16**.

Figure: 16 Standard Curve of Rutin

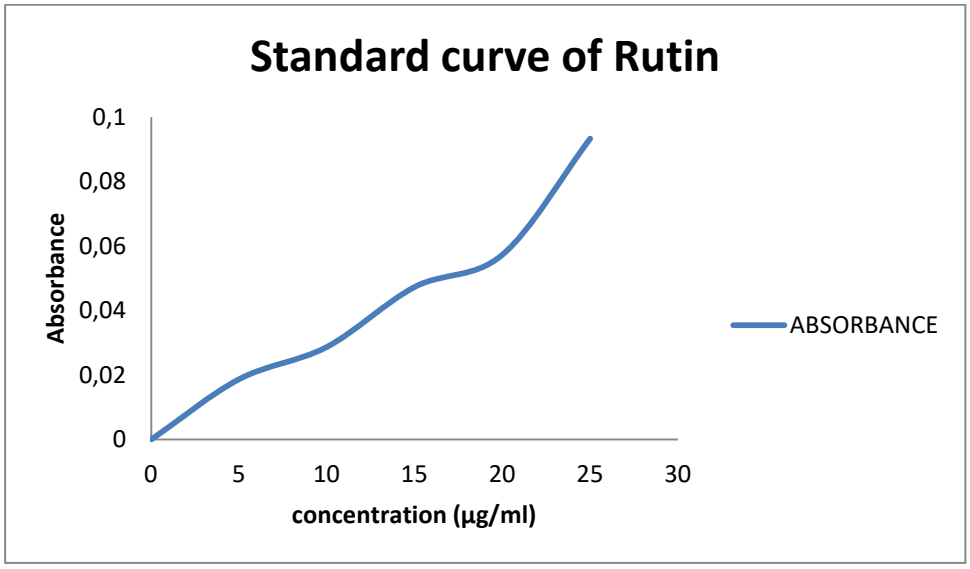


Table: 10 Determination of RutinEquivallent in HAECB

Sl.no	Concentration		Absorbance		Amount of Rutin equivalent (RE) in (HAECB) (µg/ml)
	Rutin (µg/ml)	HAECB (µg/ml)	Rutin	HAECB	
			*Mean ± SEM	*Mean ± SEM	
1	5	10	0.0187 ± 0.0006	0.0267 ± 0.0006	9.57
2	10	20	0.0287 ± 0.0003	0.0583 ± 0.0008	20.1
3	15		0.0473 ± 0.0008		
4	20		0.0573 ± 0.0012		
5	25		0.0933 ± 0.0008		
			RE		989 mg/g

*mean ± SEM

Hydroalcoholic extract of *Commelinabenghalensis L.* was found tocontain **989 mg/g** of RE and the standard curve of rutin and its results were displayed in **Table: 10 and Figure: 16**.

Determination of Chlorophyll "a", Chlorophyll "b", Total Chlorophyll and Total Carotenoid

Table: 11 Determination of Chlorophyll "a", Chlorophyll "b", Total Chlorophyll and Total Carotenoid

S.No	Wavelength	Absorbance
1	645	0.019
2	663	0.028
3	453	0.212

$$\text{Chlorophyll “a” (mg/g fresh tissue)} = \frac{12.7 \times \text{O.D at 663 nm} - 2.69 \times \text{O.D at 645}}{1000 \times 0.05}$$

$$\text{Chlorophyll “b” (mg/g fresh tissue)} = \frac{22.9 \times \text{O.D at 645 nm} - 4.68 \times \text{O.D at 663}}{1000 \times 0.05} \times 10$$

$$\text{Totalchlorophyll (mg/g fresh tissue)} = \frac{20.2 \times \text{O.D at 645 nm} + 8.02 \times \text{O.D at 663}}{1000 \times 0.05} \times 10$$

Total carotenoid (mg/g fresh tissue) = $0.216 \times \text{O.D 663} - 1.22 \times \text{O.D 645} - 0.354 \times \text{O.D at 663} + \text{O.452} \times \text{O.D 453}$

Table:12Amount of Chlorophyll “a”, Chlorophyll “b”, Total Chlorophyll and Total Carotenoid

Sl.no	Components	Amount
1	Chlorophyll a	0.00311 mg/gm
2	Chlorophyll b	0.017506 mg/gm
3	Total chlorophyll	0.052588 mg/gm
4	Total carotenoid	0.06878 mg/gm

ISOLATON OF LUTEIN

Lutein was isolated by using diethyl ether and methanol followed by saponification process.

UV SPECTRAL STUDIES

Isolated compound was subjected to UV spectral studies for identification of compounds

UV SPECTRAL STUDIES

Lutein and Chlorophyll

Aliquot of crude extract was transferred to cuvette (quartz) and was scanned under UV range from 300 – 700 nm in the UV – Visible spectrophotometer to check the presence of chlorophyll and lutein. It shows the absorbance maxima at 665, 466, 412 nm. It indicates the presence of chlorophyll (665 nm) and lutein (466, 412 nm)

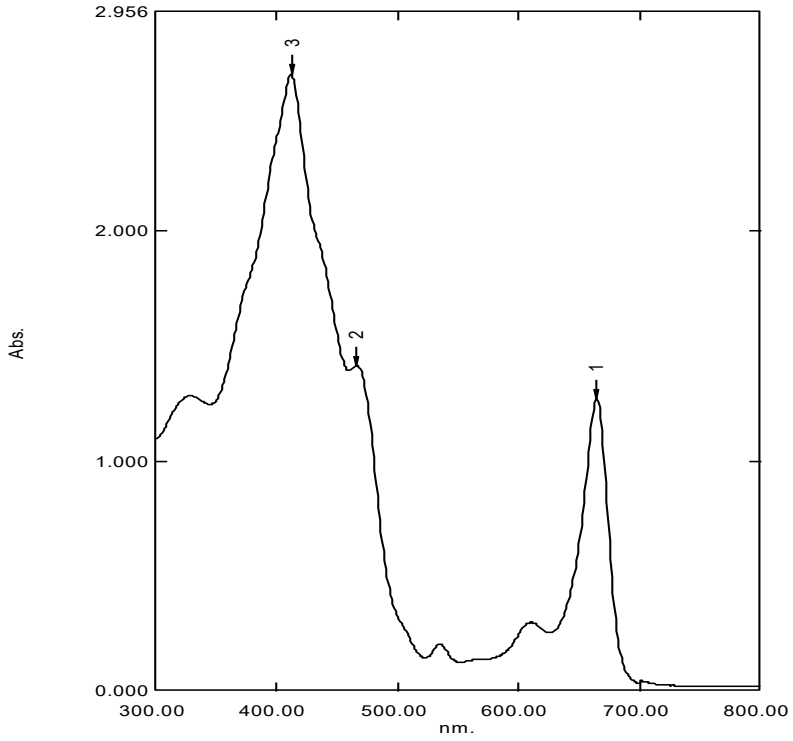


Figure: 17 UVSpectrum of Lutein and Chlorophyll

Table: 13 UV Absorbanceof proposedlutein and chlorophyll

Sl. No	WAVELENGTH (nm)	MAXIMUM ABSORBANCE MAXIMA
1	665	1.274
2	466	1.417
3	412	2.689

UV spectral studies (Shimadzu 1800)

LUTEIN

The isolated compound lutein was dissolved in diethyl ether and transferred to cuvette (quartz) and was scanned under UV range from 300 – 700 nm in the UV – Visible spectrophotometer. The isolated compound lutein shows absorbance maxima at 470, 444 nm.

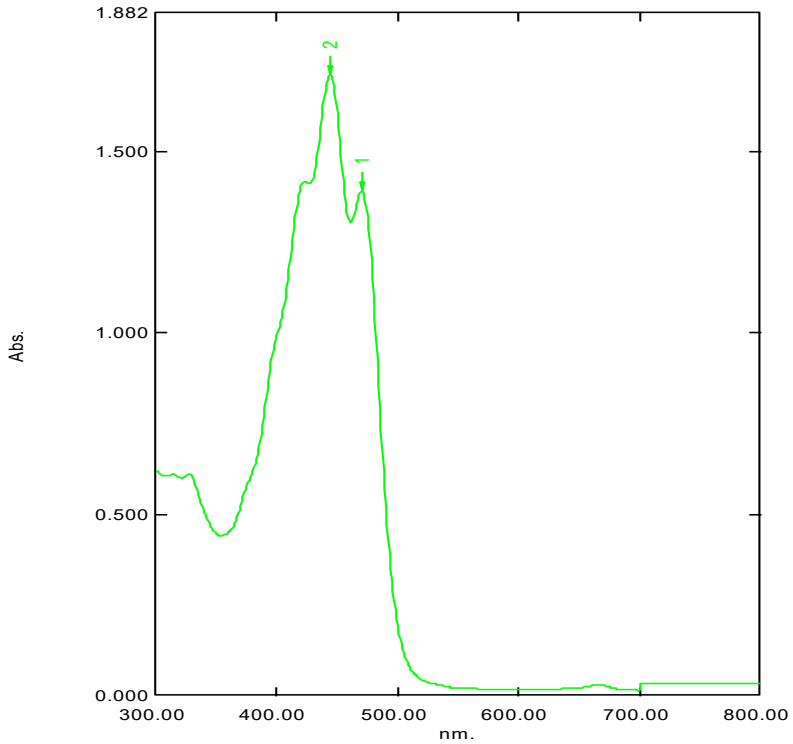


Figure: 18 UV Spectrum of Proposed Lutein

Table: 14 UV Absorbance of proposed lutein

Sl.no	WAVELENGTH (nm)	MAXIMUM ABSORBANCE MAXIMA
1	470	1.392
2	444	1.712

^{1}H NMR SPECTRUM OF PROPOSED LUTEIN

The Isolated compound was dissolved in Deuterated chloroform ($CDCL_3$) and the NMR spectrum was obtained by using UXNMR BrukerAnalytischeMesstechnikGmbh.

^{1}H NMR

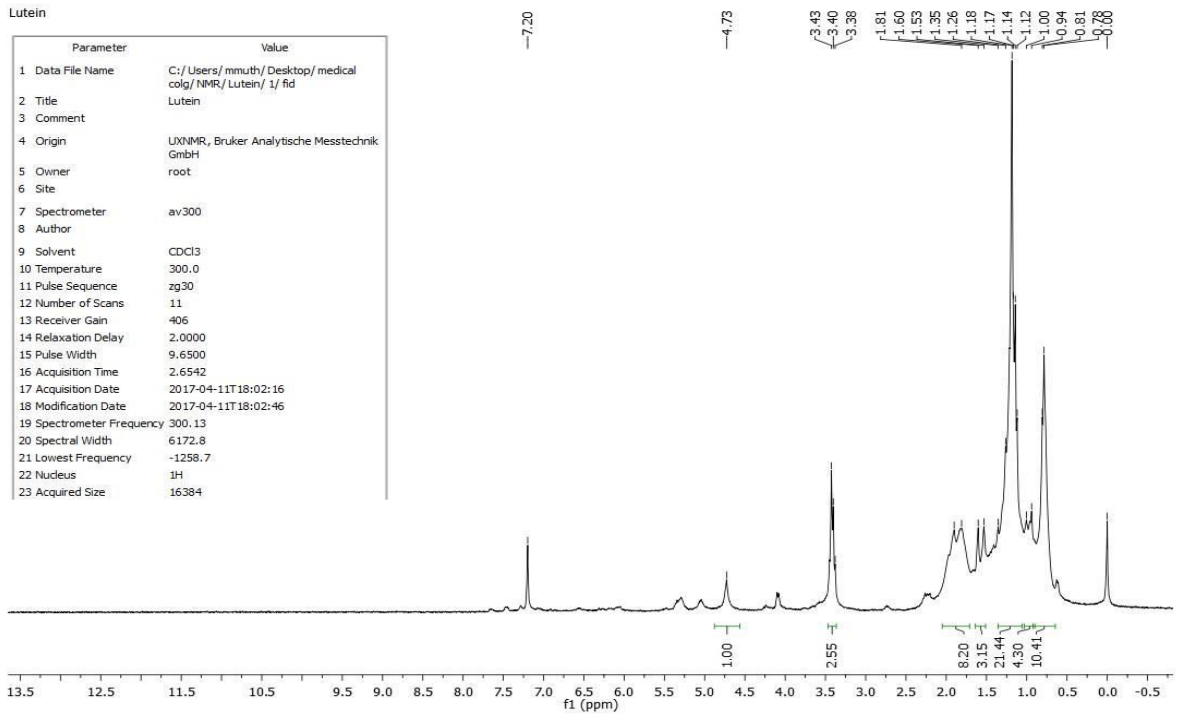


Figure: 19 ^{1}H Nmr Spectrum of Proposed Lutein

Chemical shift values.

^{1}H NMR: (300 MHz, $CDCl_3$) δ_H 0.78(s, 10H), 0.94 - 1.00 (bd, 2H), 1.12 - 1.35 (bm, 21H), 1.53-1.60 (bd, 3H), 1.81-1.90 (bd, 8H), 3.38-3.45 (m, 2H), 4.73 (s, 1H).

Table: 15 Observed chemical shift value of ^{1}H NMR

Sl.No	δvalue	Band name	Position of hydrogen atom
1	0.78	s	10H
2	0.94 - 1.00	bd	2H
3	1.12 - 1.35	bm	21H
4	1.53-1.60	bd	3H
5	1.81-1.90	bd	8H
6	3.38-3.45	m	2H
7	4.73	s	1H

Table: 16Reported chemical shift value of ^{1}H NMR

Sl.No	δvalue	Band name	Position of hydrogen atom
1	0.998	s	6H
2	1.074	S	6H
3	1.37	Dd	1H
4	1.626	S	3H
5	1.739	S	3H
6	1.84	dd	1H
7	1.912	s	3H

8	1.970	s	9H
9	2.33-2.45	m	2H
10	4.0	m	1H

^{13}C NMR SPECTRUM OF PROPOSED LUTEIN

The Isolated compound was dissolved in Duterated chloroform ($CDCL_3$) and the NMR spectrum was obtained by using UXNMR BrukerAnalytischeMesstechnikGmbh.

^{13}C NMR

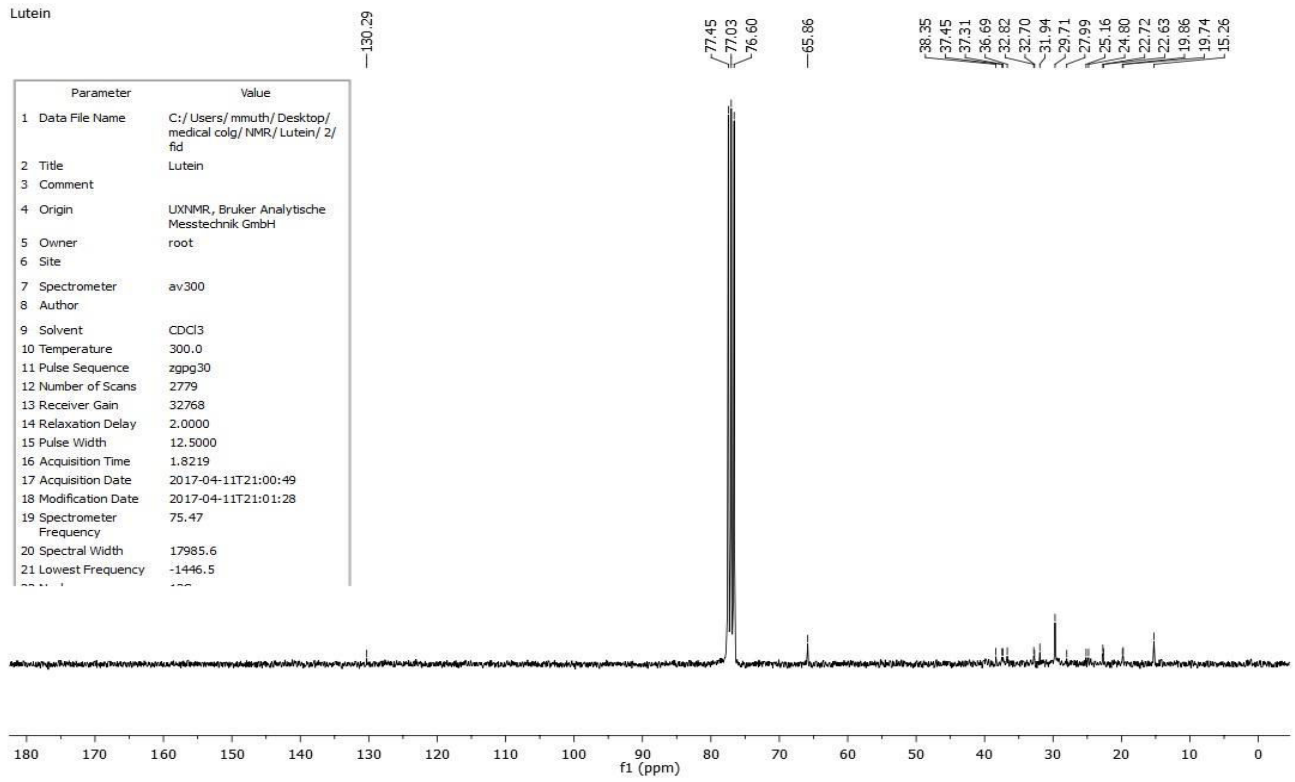


Figure: 20 ^{13}C NMR Spectrum of Proposed Lutein

Chemical shift values.

^{13}C NMR: (75 MHz, $CDCl_3$); δ_C 15.3, 19.7, 19.9, 22.6, 22.7, 24.8, 25.2, 28.0, 29.7, 31.9, 32.7, 32.8, 36.7, 37.3, 37.5, 38.4, 65.9, 130.3.

Table: 17 CHEMICAL SHIFT VALUE OF ^{13}C NMR

Sl.No	CHEMICAL SHIFT VALUE OF ^{13}C NMR OBSERVED	CHEMICAL SHIFT VALUE OF ^{13}C NMR REPORTED
1	15.3	13.2
2	19.7	21.6
3	19.9	22.8
4	22.6	24.3
5	22.7	28.7
6	24.8	29.5
7	25.2	30.2
8	28.0	34.0
9	29.7	37.1
10	31.9	65.9
11	32.7	130.0
12	32.8	
13	36.7	
14	37.3	
15	37.5	
16	38.4	
17	65.9	
18	130.3	

GC-MS STUDIES

The isolated compound was subjected to GC-MS studies and the spectrum was obtained by using bruker instrument.

GC-MS PROFILE OF PROPOSED LUTEIN

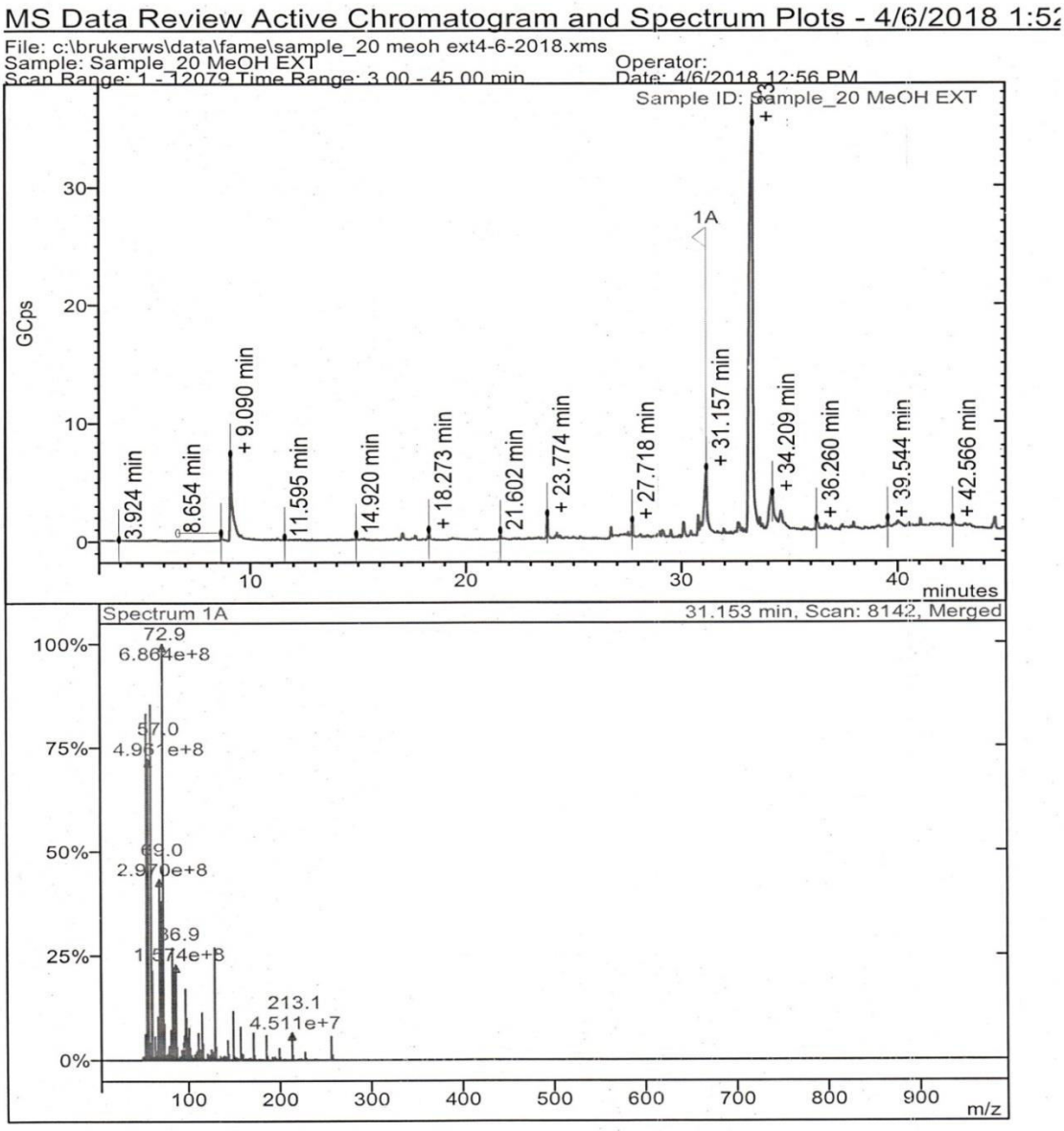


Figure: 21 GC-MS Profile of Proposed Lutein

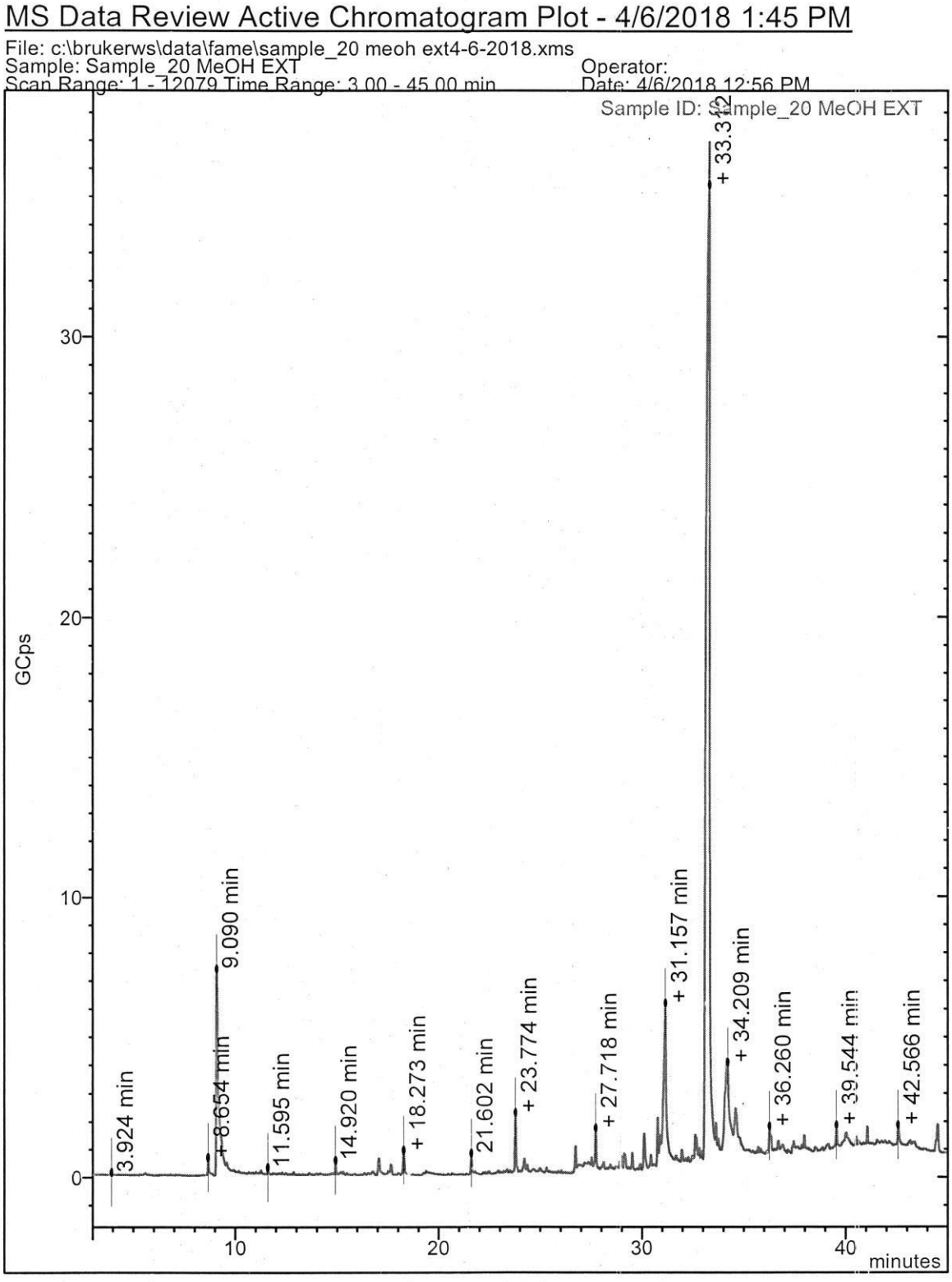


Figure: 21.1 GC-MS Profile of Proposed Lutein

GC-MS PROFILE

Lock Peak Width:	Yes
Parameters:	Local
Peak Width (sec):	50.0
Slope Sensitivity (SN):	25
Tangent %:	20
Peak Size Reject (counts):	2000
Smoothing:	Mean 7 Point Smooth
Spike Threshold Factor:	10
Noise:	Peak to Peak

	Retention Time	Area	% of Total	Signal/Noise	Scan Description
1.	7.249	110051536	0.013	18.35	Merged
2.	8.899	7.040e+10	8.312	-2.707	Merged
3.	16.608	4.086e+9	0.482	10.27	Merged
4.	17.939	7.482e+9	0.883	0.6828	Merged
5.	21.356	2.855e+9	0.337	14.7	Merged
6.	21.850	2.215e+9	0.262	22.79	Merged
7.	23.567	1.174e+10	1.386	4.836	Merged
8.	23.847	5.956e+9	0.703	25.08	Merged
9.	24.403	5.003e+9	0.591	9.307	Merged
10.	27.230	1.814e+10	2.142	20.16	Merged
11.	29.167	5.118e+9	0.604	38.93	Merged
12.	30.787	1.895e+10	2.237	83.71	Merged
13.	31.304	5.861e+10	6.921	44.45	Merged
14.	32.947	4.271e+11	50.426	9.485	Merged
15.	33.952	1.204e+11	14.213	10.42	Merged
16.	36.079	2.218e+10	2.619	12.66	Merged
17.	37.549	1.880e+10	2.219	20.7	Merged
18.	39.767	2.110e+10	2.492	16.46	Merged
19.	40.209	1.193e+10	1.409	28.24	Merged
20.	42.491	1.481e+10	1.749	16.15	Merged

Figure: 21.2 GC-MS Profile of Proposed Lutein

PEAK 1

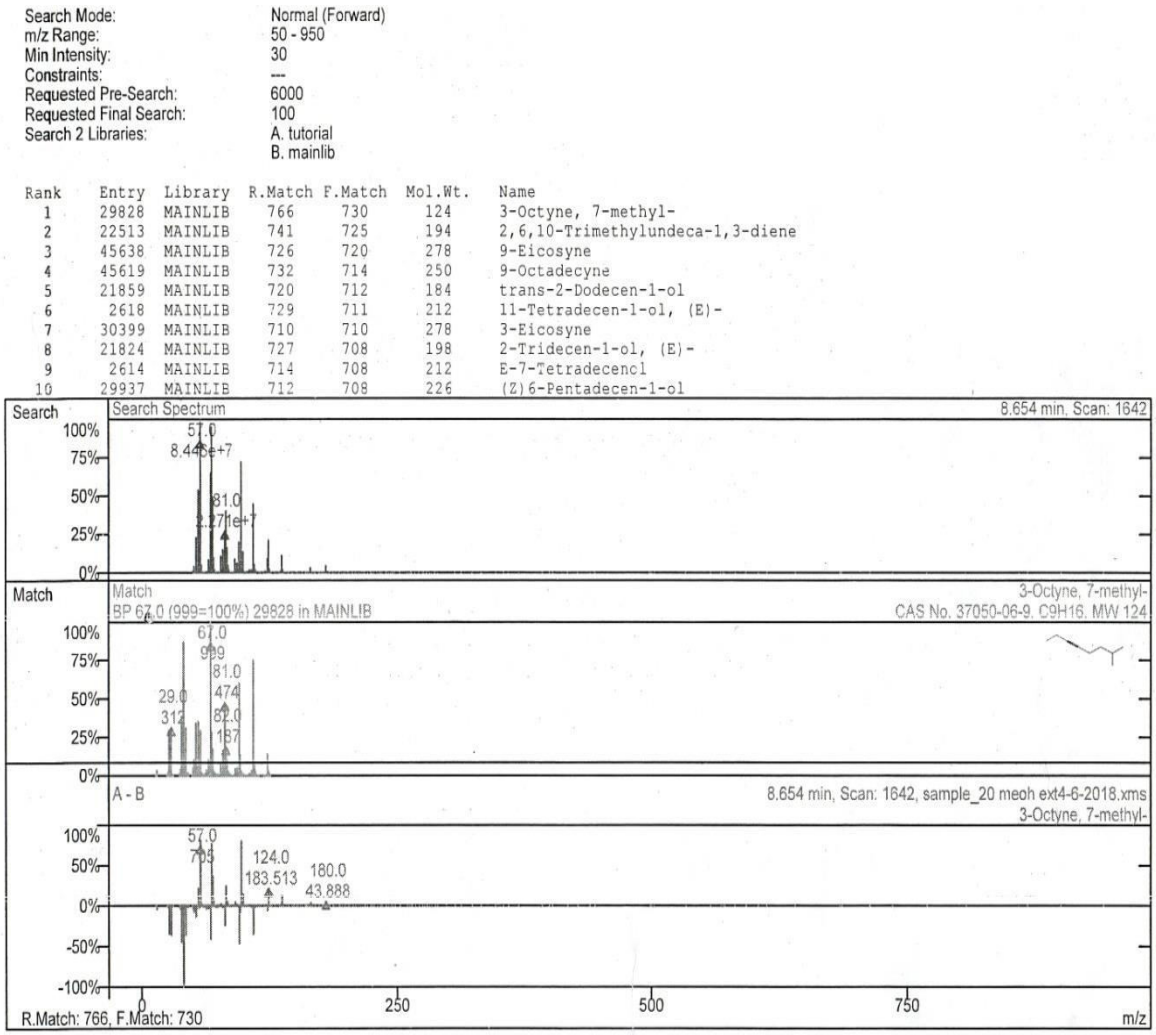


Figure:21.3 GC-MS profile of proposed lutein

PEAK 2

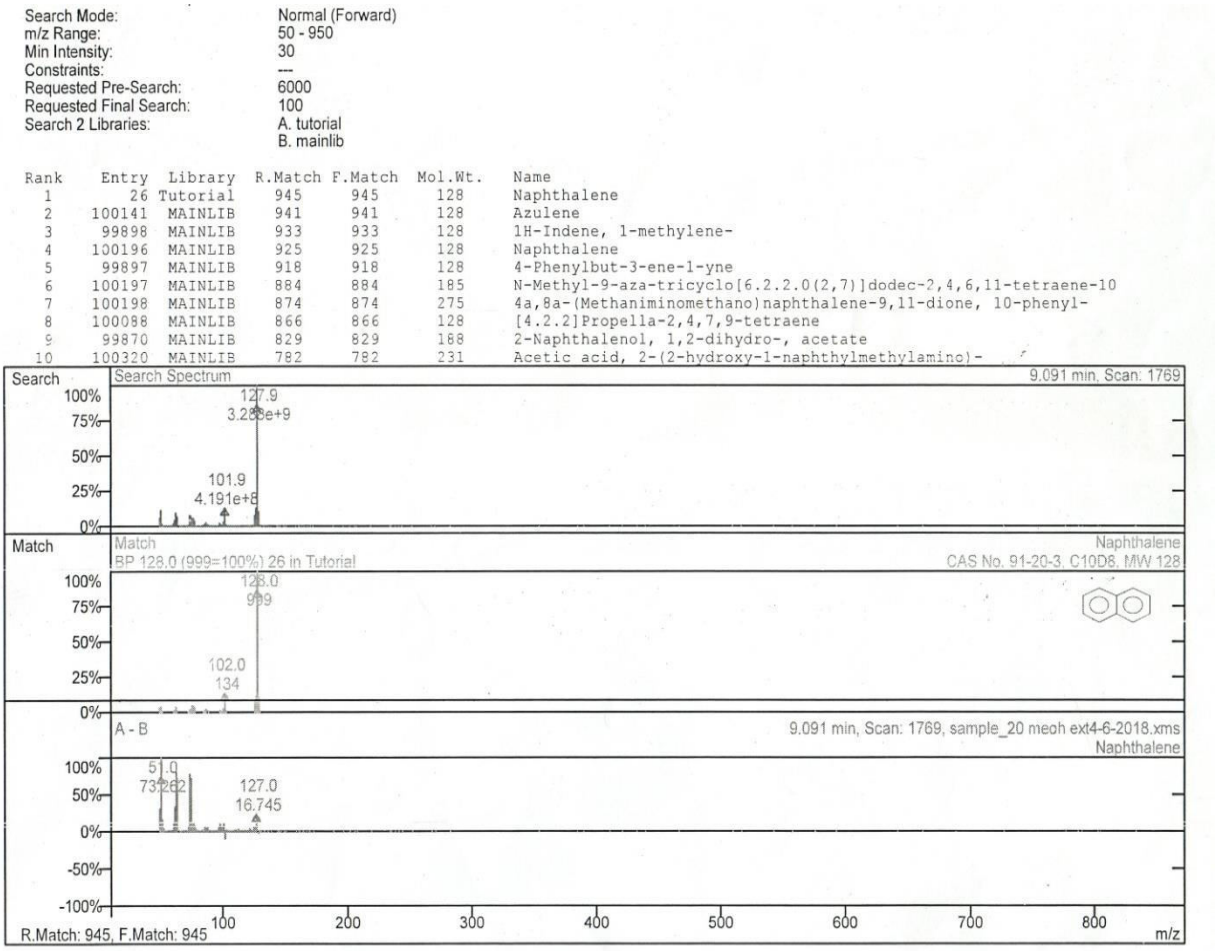


Figure: 21.4 GC-MS profile of proposed lutein

PEAK 3

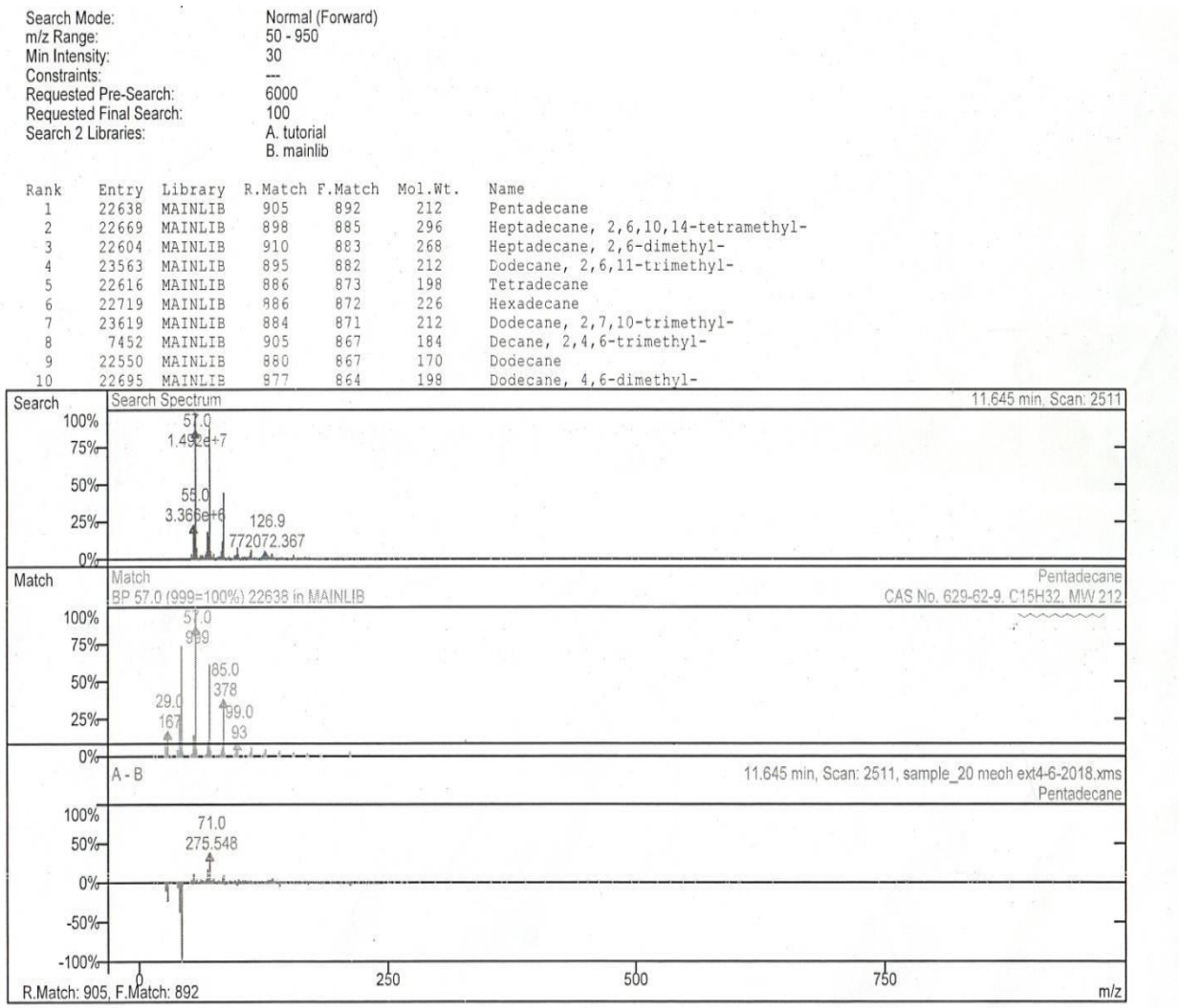


Figure: 21.5 GC-MS profile of proposed lutein

PEAK 4

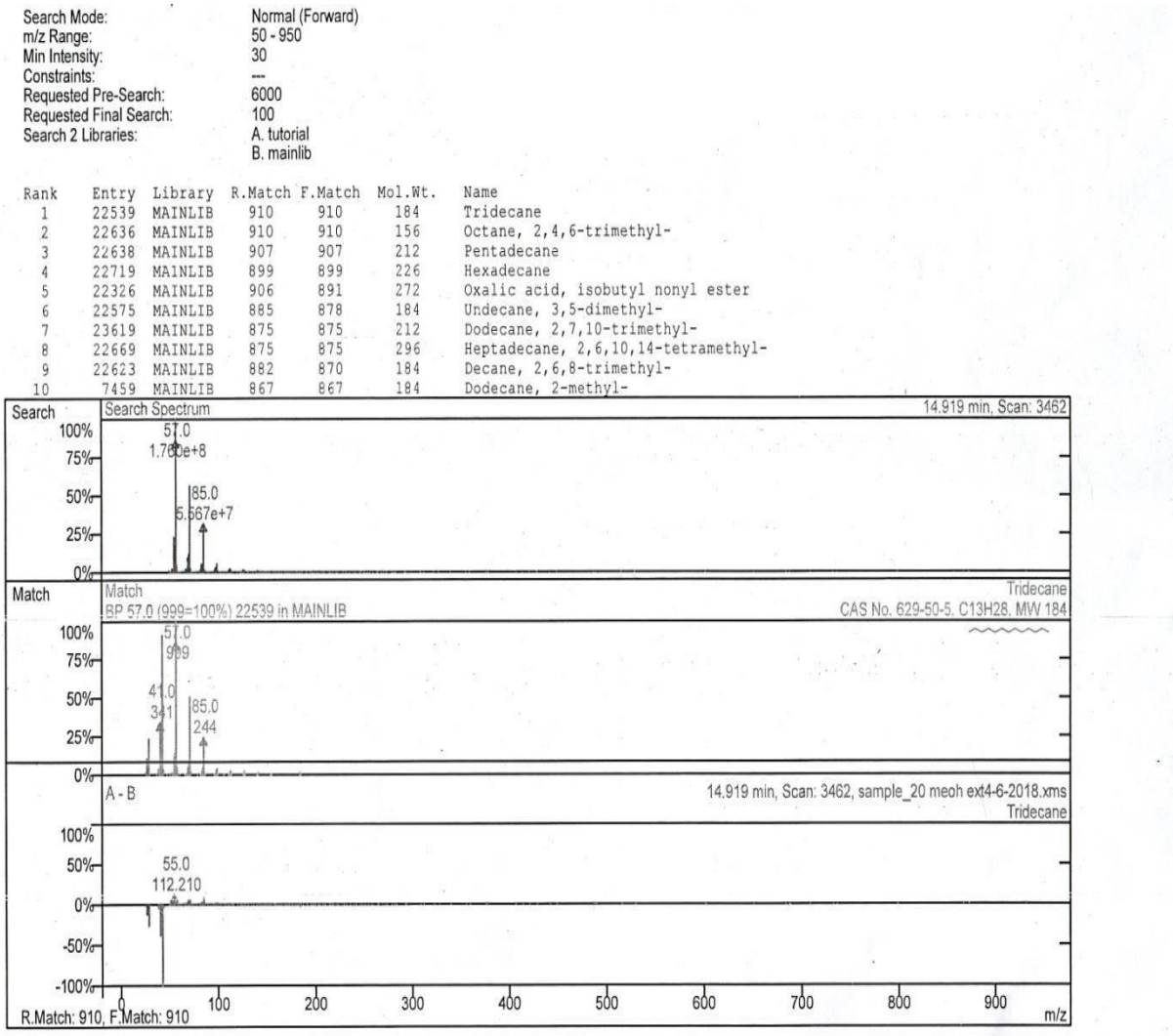


Figure: 21.6 GC-MS profile of proposed lutein

PEAK 5

Top Ten Summary of Search NIST Libraries for Spectrum

Search NIST Libraries for Spectrum Results
Hits Found: 100

Search NIST Libraries for Spectrum Parameters
Search Mode: Normal (Forward)
m/z Range: 50 - 950
Min Intensity: 30
Constraints: ---
Requested Pre-Search: 6000
Requested Final Search: 100
Search 2 Libraries: A. tutorial
B. mainlib

Rank	Entry	Library	R.Match	F.Match	Mol.Wt.	Name
1	165050	MAINLIB	926	924	220	Butylated Hydroxytoluene
2	165051	MAINLIB	856	830	277	Phenol, 2,6-bis(1,1-dimethylethyl)-4-methyl-, methylcarbamate
3	165048	MAINLIB	827	820	220	Phenol, 4,6-di(1,1-dimethylethyl)-2-methyl-
4	165543	MAINLIB	803	803	304	Cyclopropanecarboxylic acid, 1-hydroxy-, (2,6-di-t-butyl-4-meth
5	165044	MAINLIB	790	790	220	4,6-di-tert-Butyl-m-cresol
6	165024	MAINLIB	777	777	220	Ethanone, 1-(5,6,7,8-tetrahydro-2,8,8-trimethyl-4H-cyclchepta[b
7	165542	MAINLIB	772	767	262	2,6-Di-t-butyl-4-methylphenol acetate(ester)
8	165557	MAINLIB	769	767	220	Phenol, 2,4,6-tris(1-methylethyl)-
9	165547	MAINLIB	719	719	220	Propanoic acid, 2-methyl-3-[4-t-butyl]phenyl-
10	172567	MAINLIB	712	712	220	Benzo[b]dihydropyran, 6-hydroxy-4,4,5,7,8-pentamethyl-

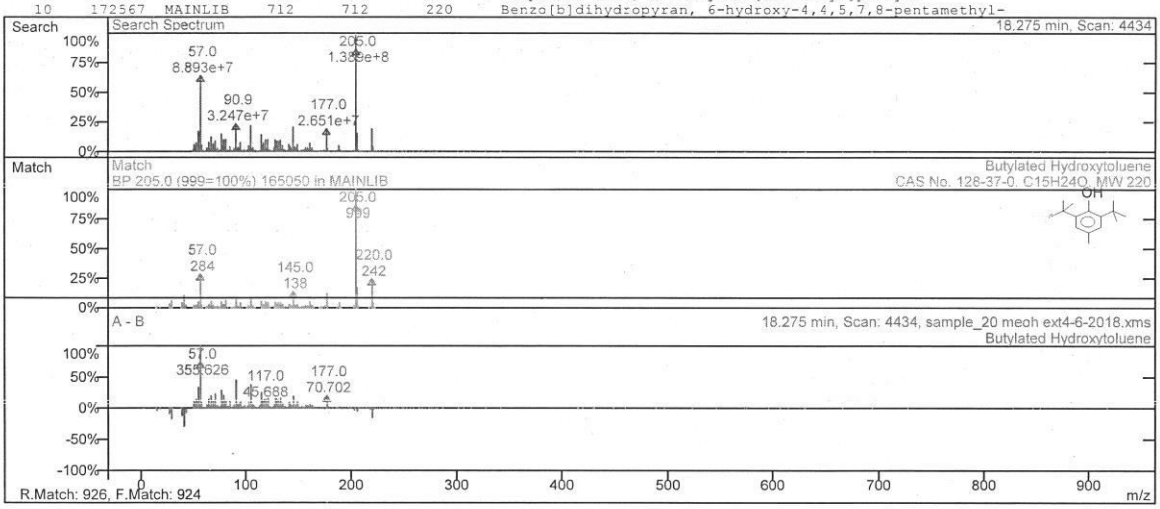


Figure: 21.7 GC-MS profile of proposed lutein

PEAK 6

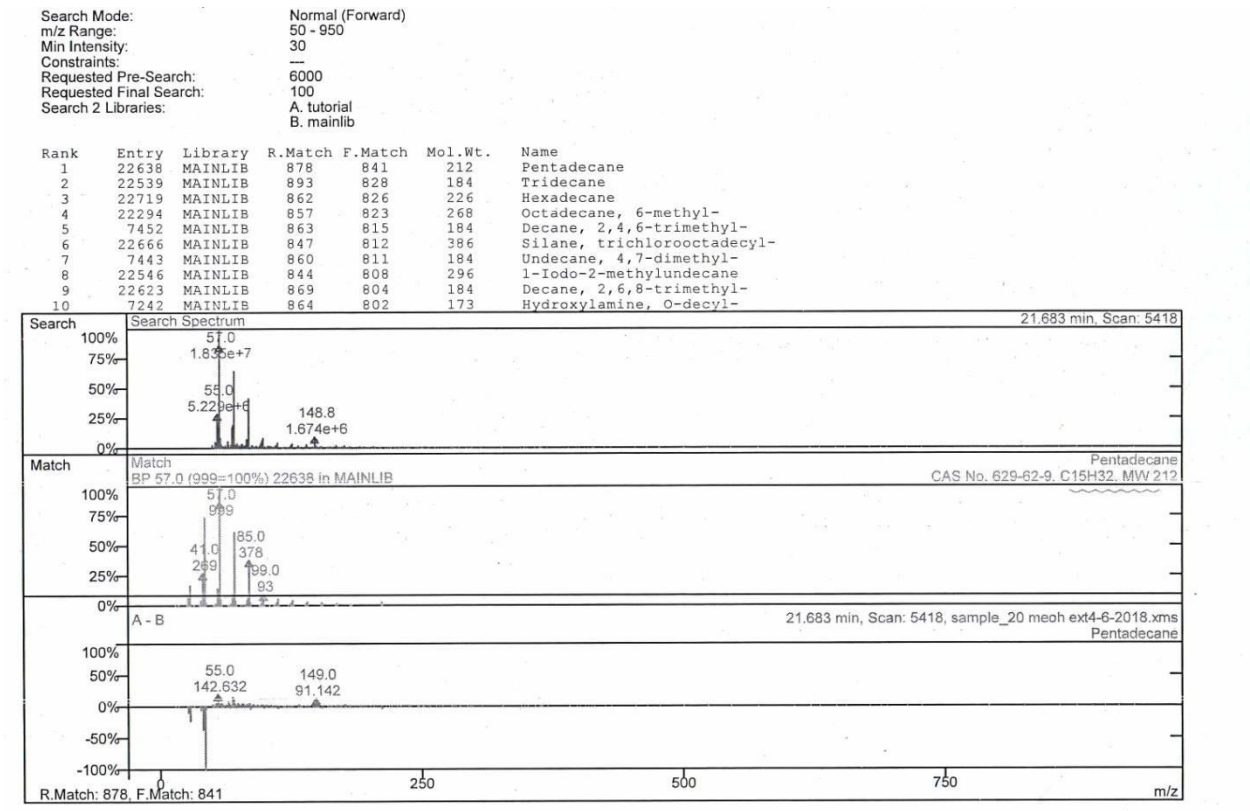


Figure: 21.8 GC-MS profile of proposed lutein

PEAK 7

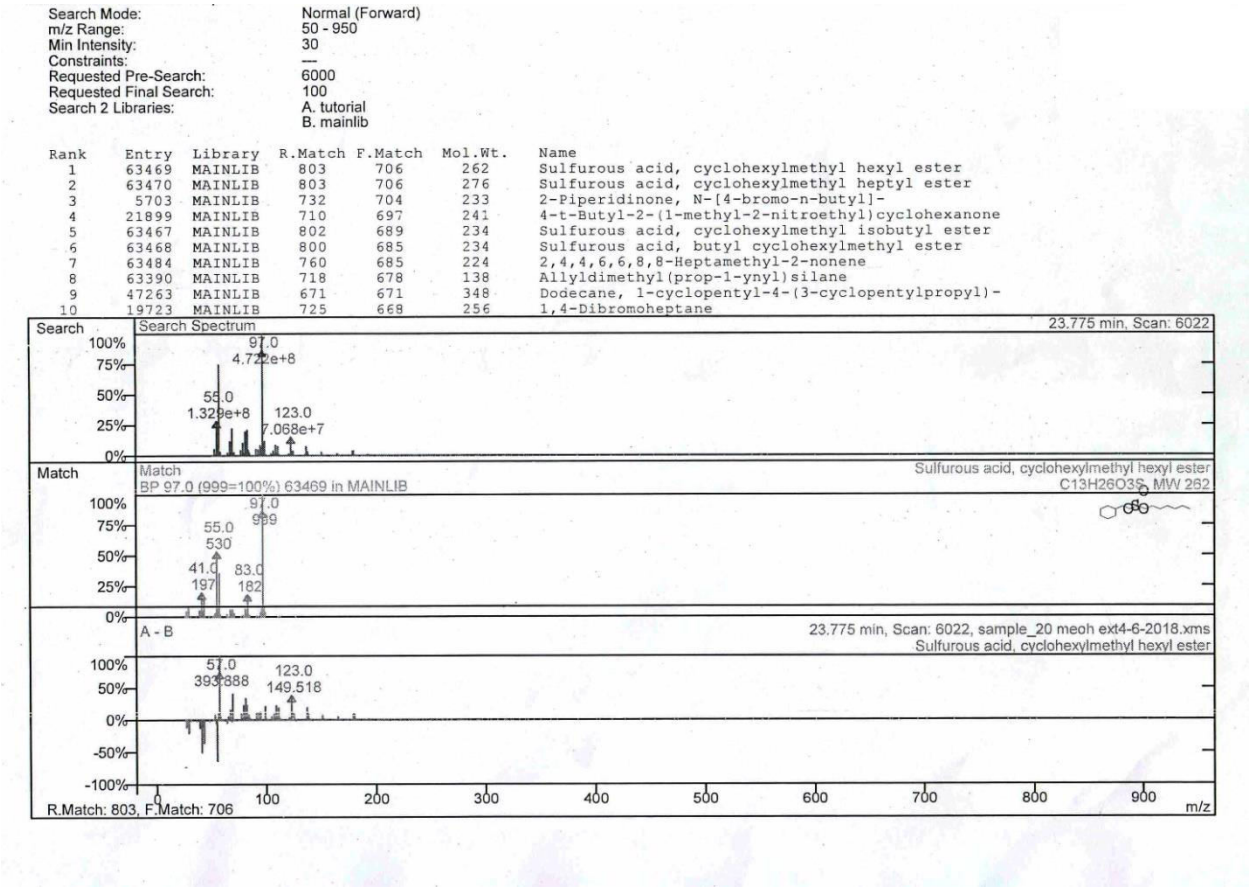


Figure: 21.9 GC-MS profile of proposed lutein

PEAK 8

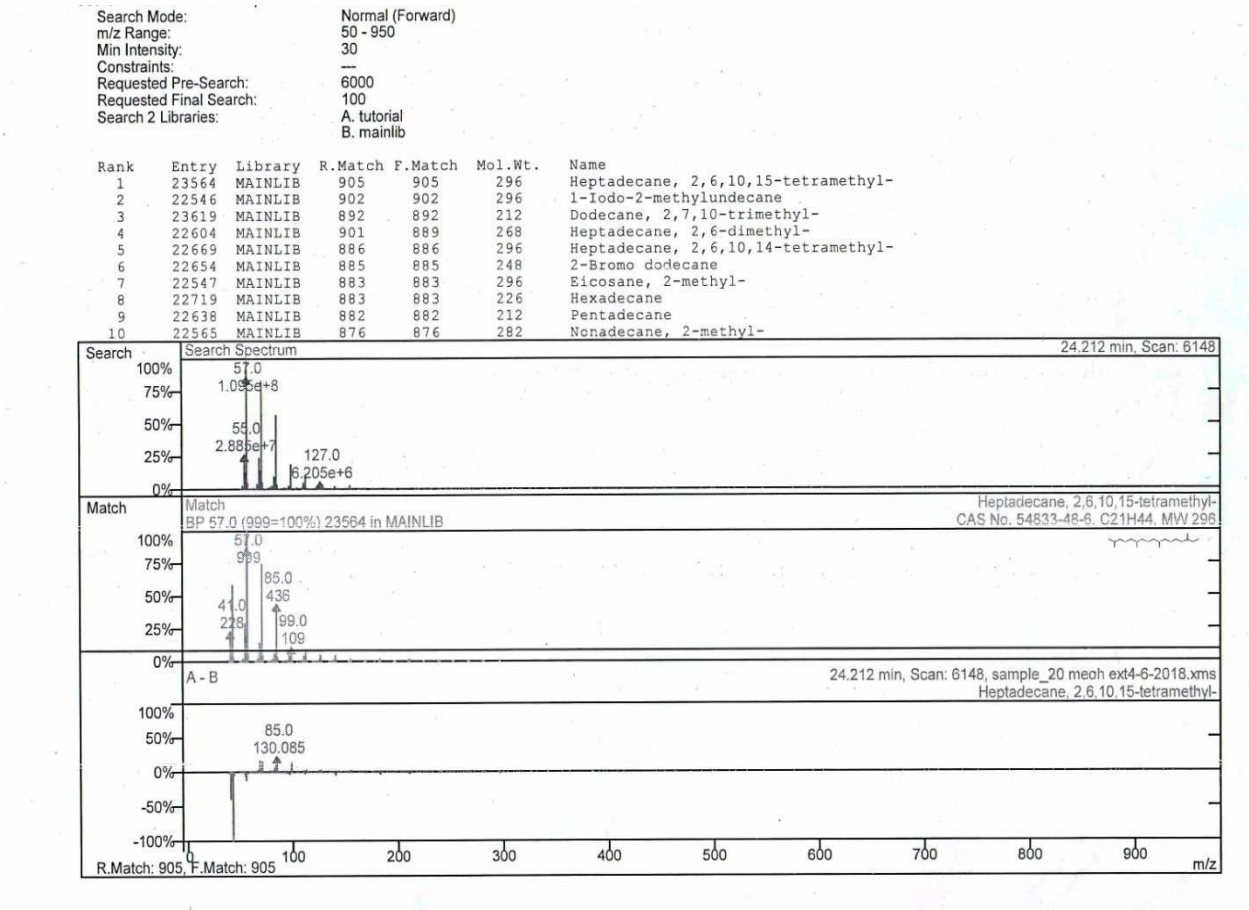


Figure: 21.10 GC-MS profile of proposed lutein

PEAK 9

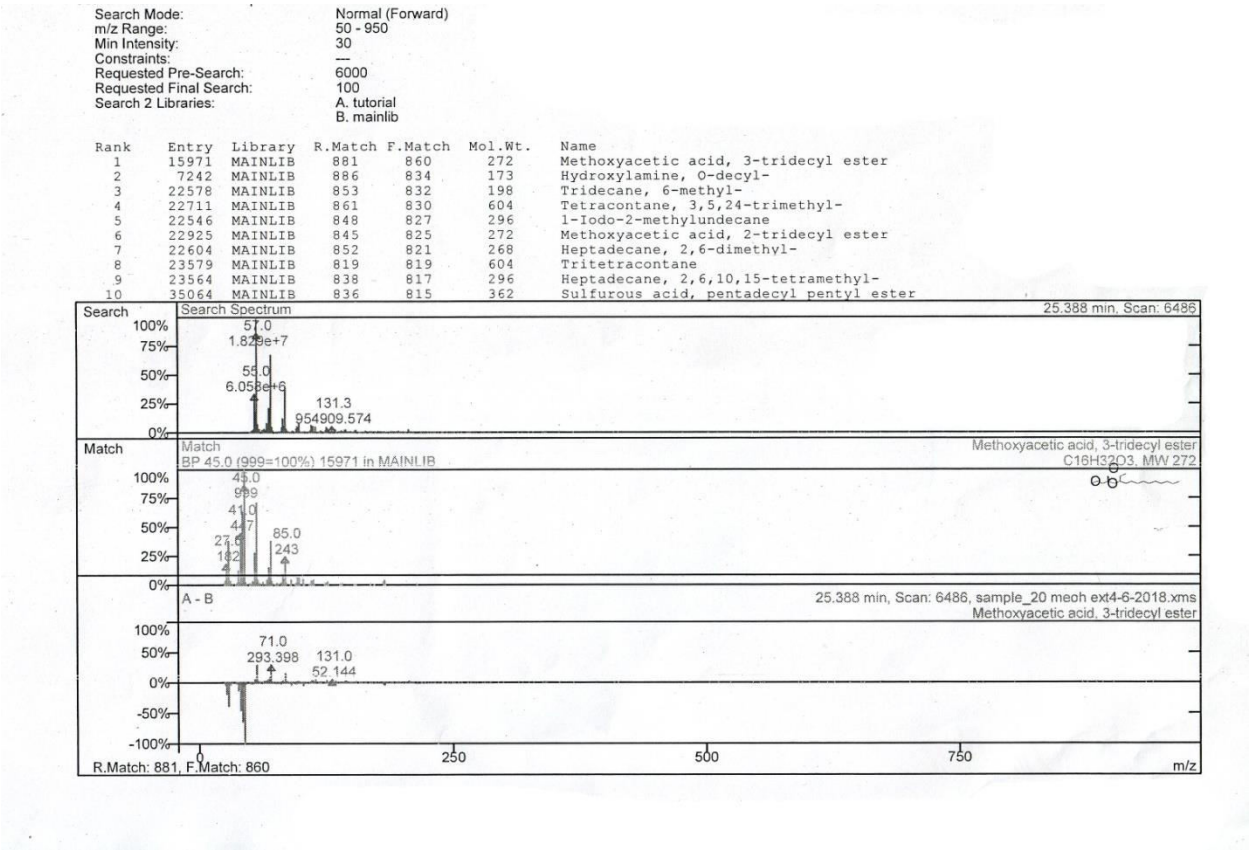


Figure: 21.11 GC-MS profile of proposed lutein

PEAK 10

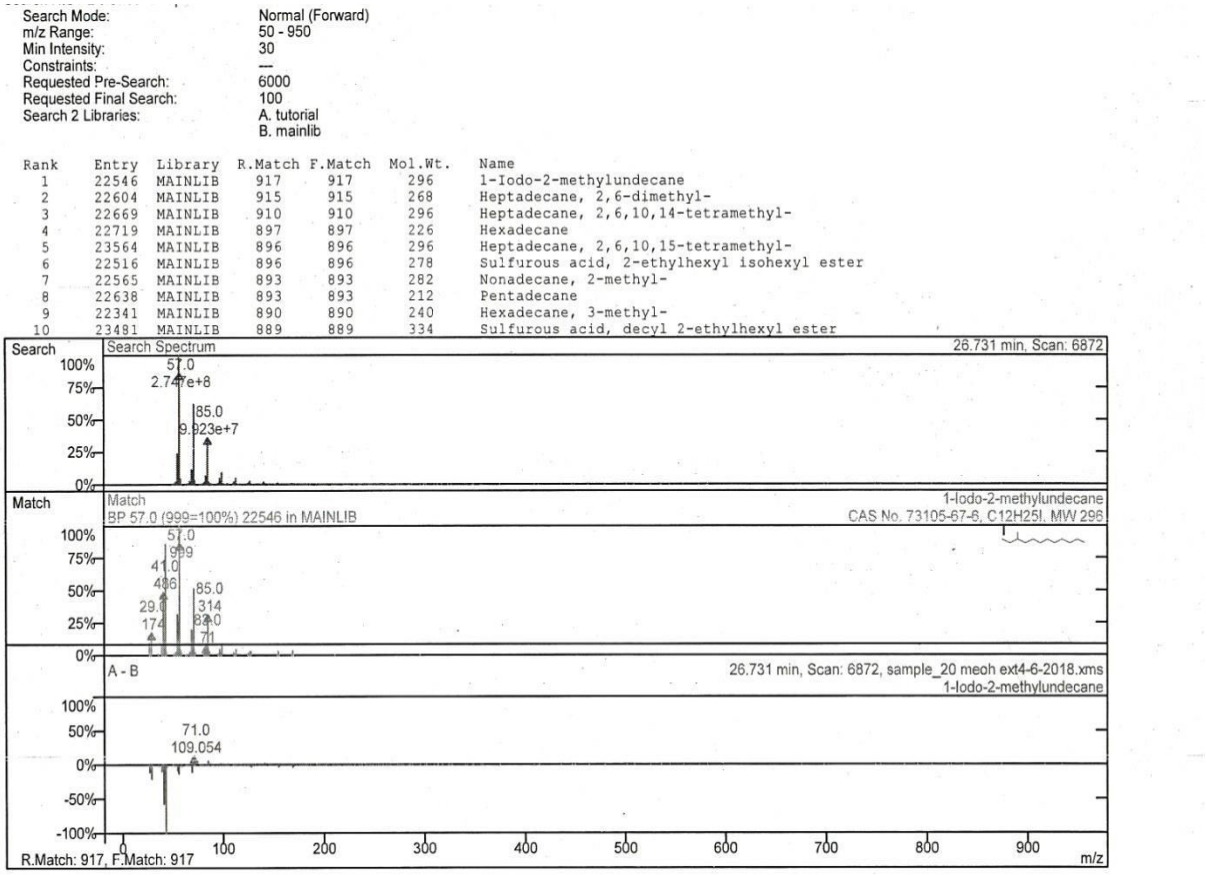


Figure: 21.12 GC-MS profile of proposed lutein

PEAK 11

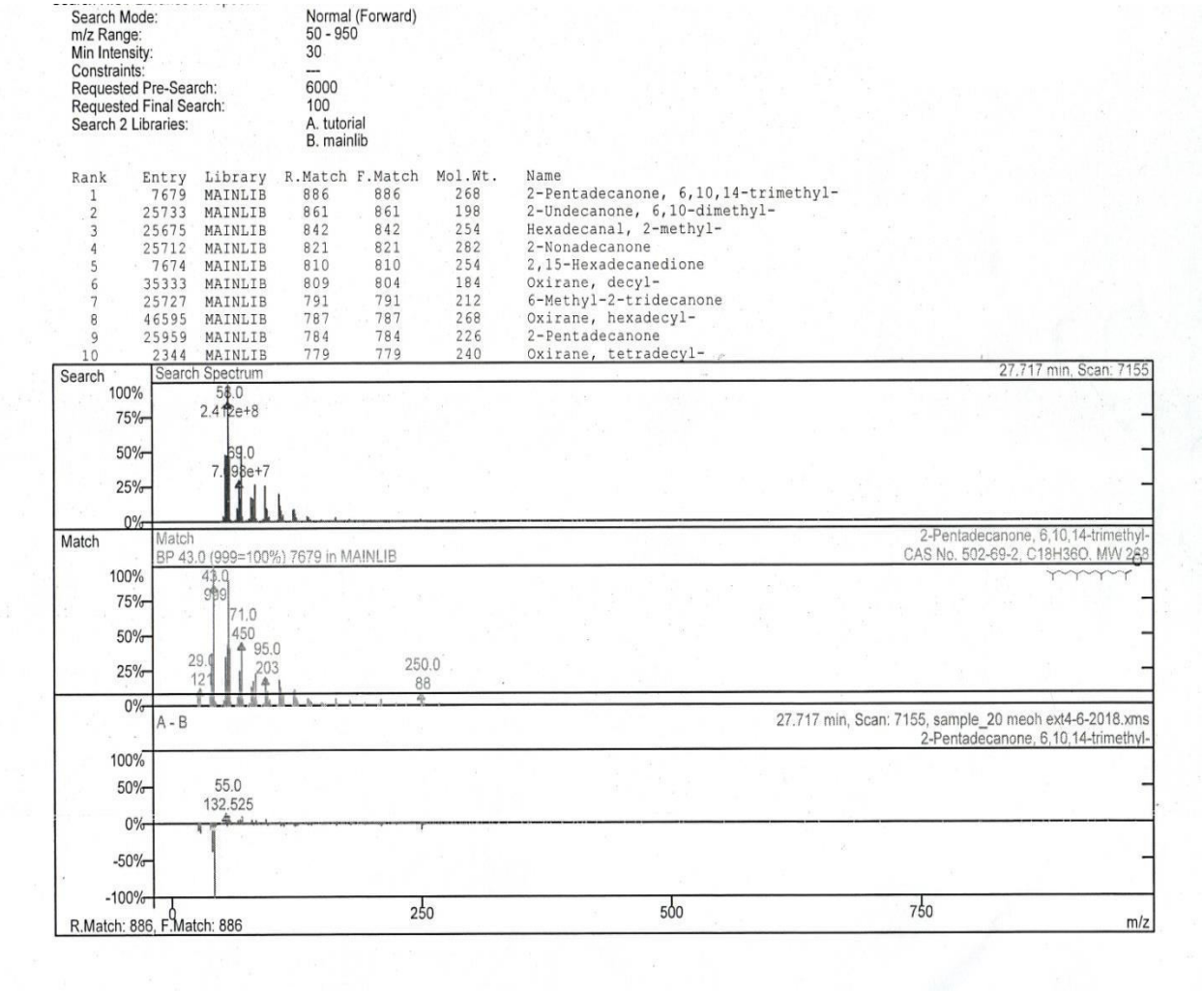


Figure: 21.13 GC-MS profile of proposed lutein

PEAK 12

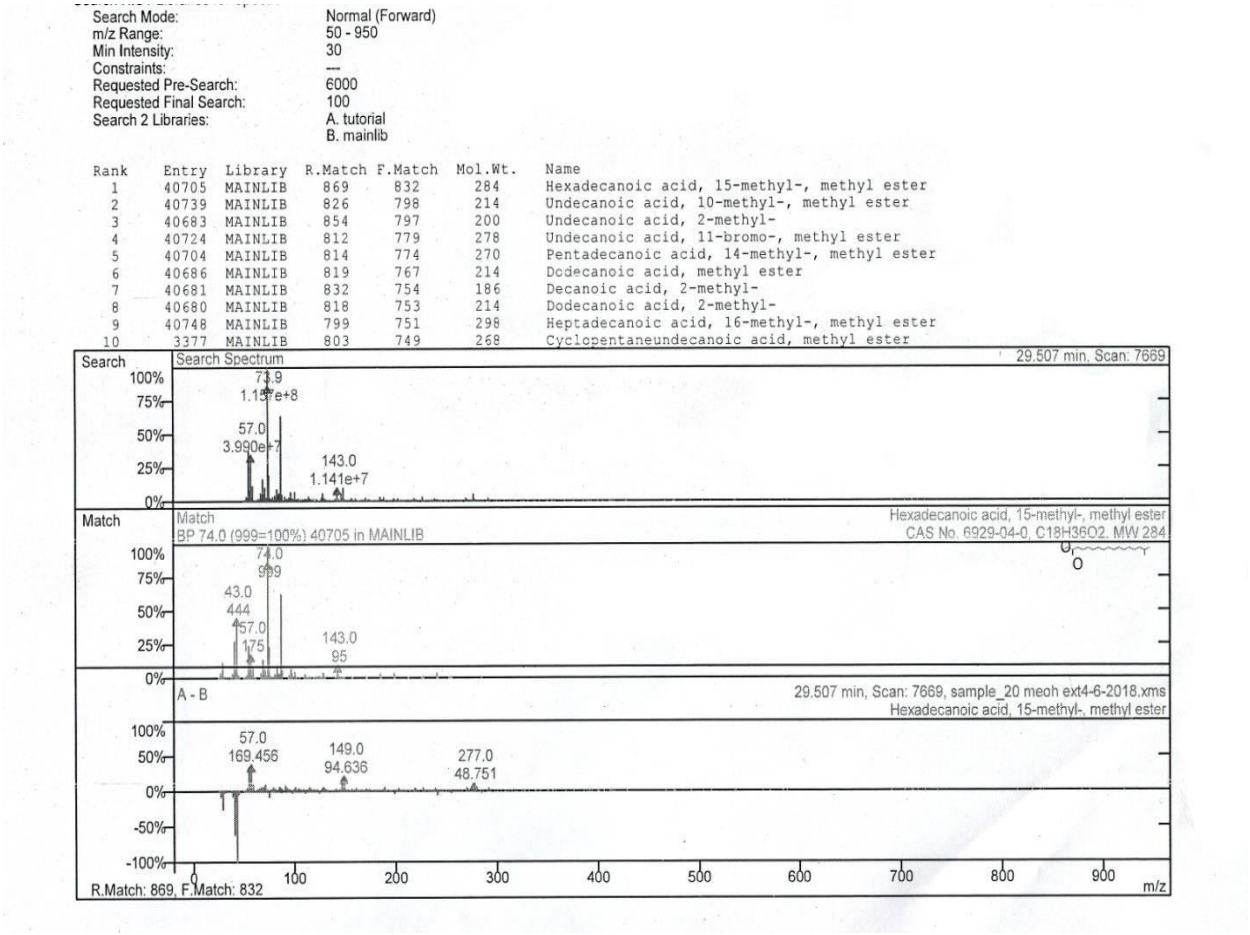


Figure: 21.14 GC-MS profile of proposed lutein

PEAK 13

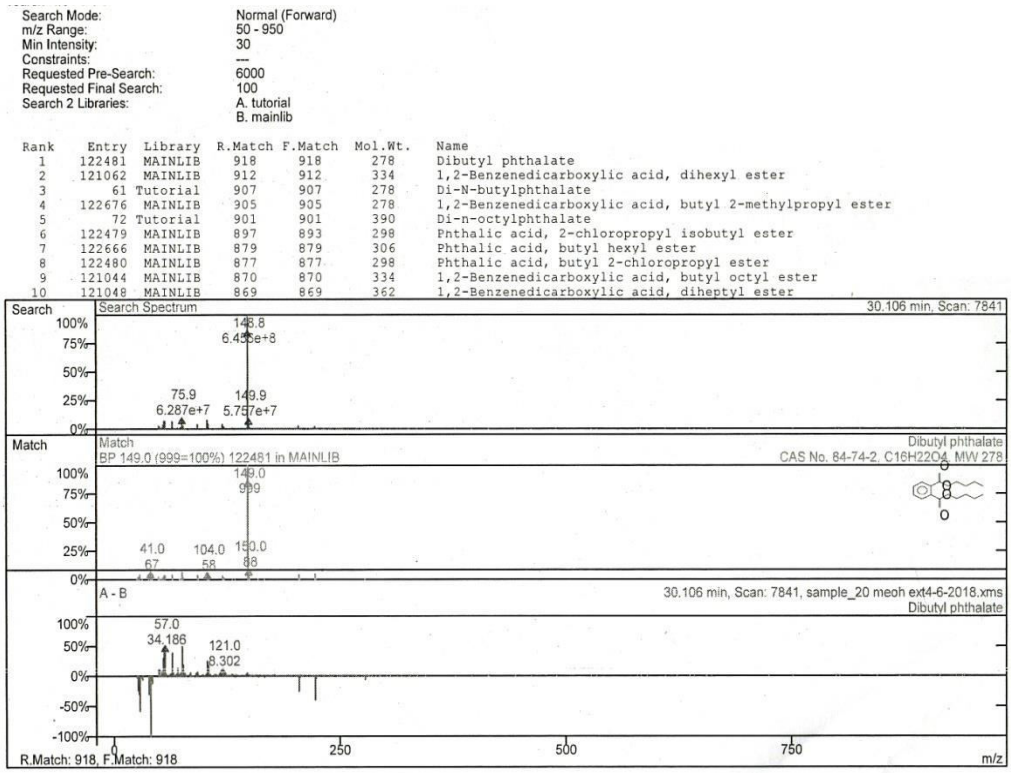


Figure:21.15 GC-MS profile of proposed lutein

PEAK 14

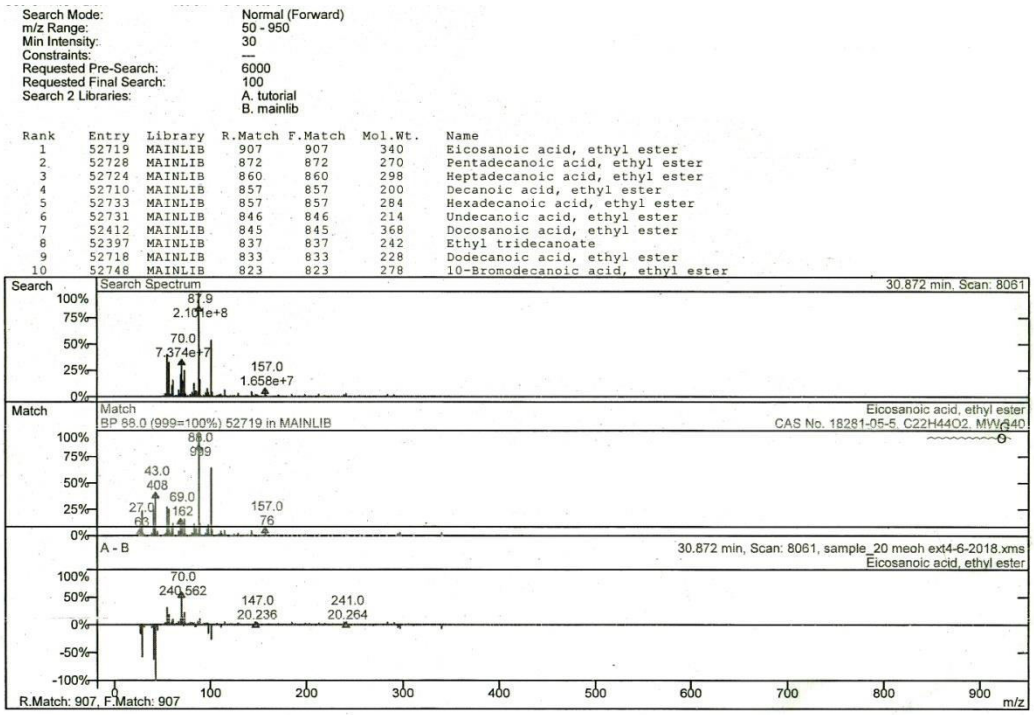


Figure: 21.16 GC-MS profile of proposed lutein

PEAK 15

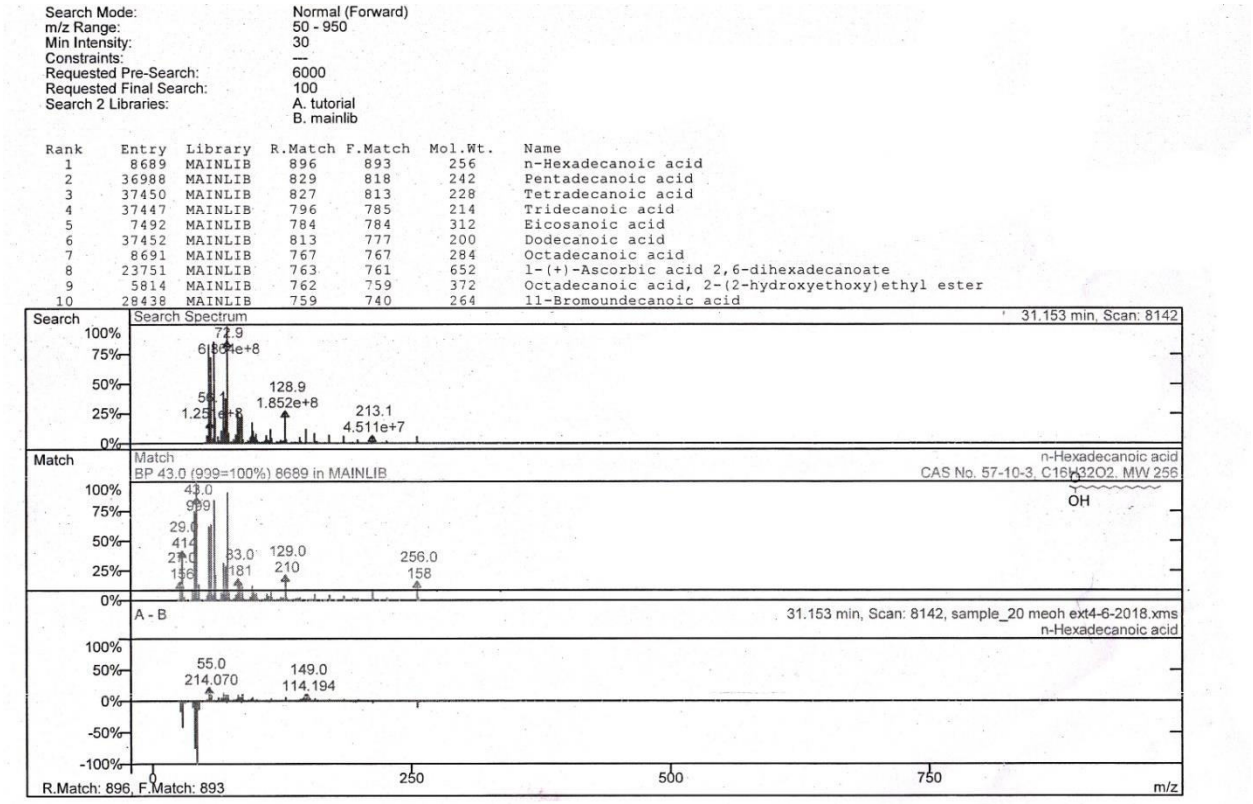


Figure:21.17 GC-MS profile of proposed lutein

PEAK 16

Top Ten Summary of Search NIST Libraries for Spectrum

Search NIST Libraries for Spectrum Results
Hits Found: 100

Search NIST Libraries for Spectrum Parameters
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Min Intensity: 30
Constraints: ---
Requested Pre-Search: 6000
Requested Final Search: 100
Search 2 Libraries: A. tutorial
B. mainlib

Rank	Entry	Library	R.Match	F.Match	Mol.Wt.	Name
1	67445	MAINLIB	799	799	326	Hexadecanoic acid, 3,7,11,15-tetramethyl-, methyl ester
2	40789	MAINLIB	752	750	270	Methyl 3-methyl-pentadecanoate
3	40784	MAINLIB	741	741	340	Eicosanoic acid, 3-methyl-, methyl ester
4	2255	MAINLIB	740	740	310	Cyclopropanepentanoic acid, 2-undecyl-, methyl ester, trans-
5	67448	MAINLIB	734	733	256	Dodecanoic acid, 3,7,11-trimethyl-, methyl ester
6	67387	MAINLIB	747	723	408	Succinic acid, octyl tridec-2-ynyl ester
7	66950	MAINLIB	732	717	328	Acetoxyacetic acid, 4-pentadecyl ester
8	22450	MAINLIB	722	711	298	Oxirane, [(hexadecyloxy)methyl]-
9	40782	MAINLIB	757	708	242	Tridecanoic acid, 3-methyl-, methyl ester
10	67451	MAINLIB	706	706	396	Tricosanoic acid, 3,5-dimethyl-, methyl ester, (R*,R*)-

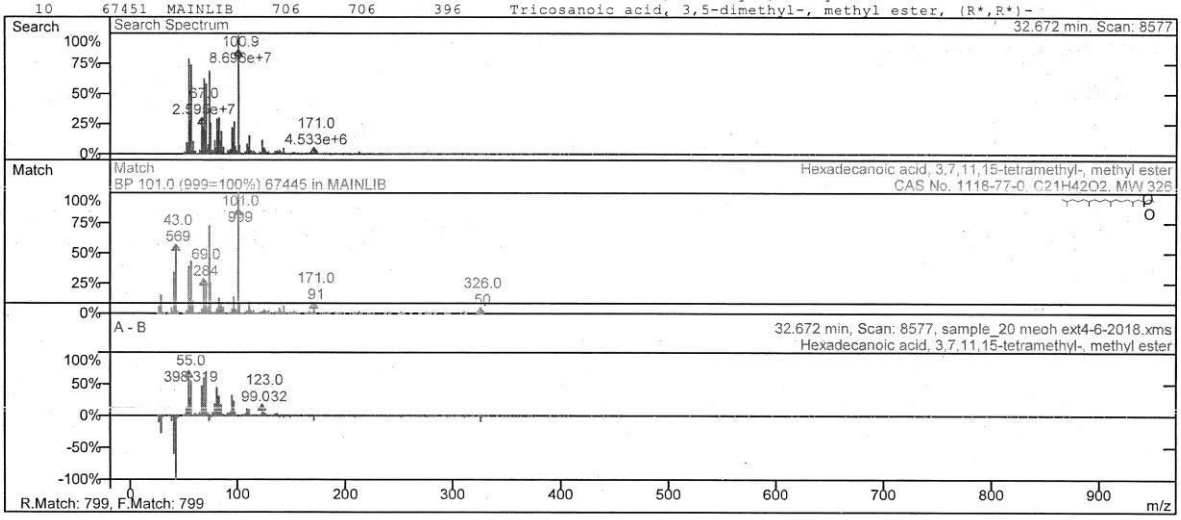


Figure: 21.18 GC-MS profile of proposed lutein

PEAK 17

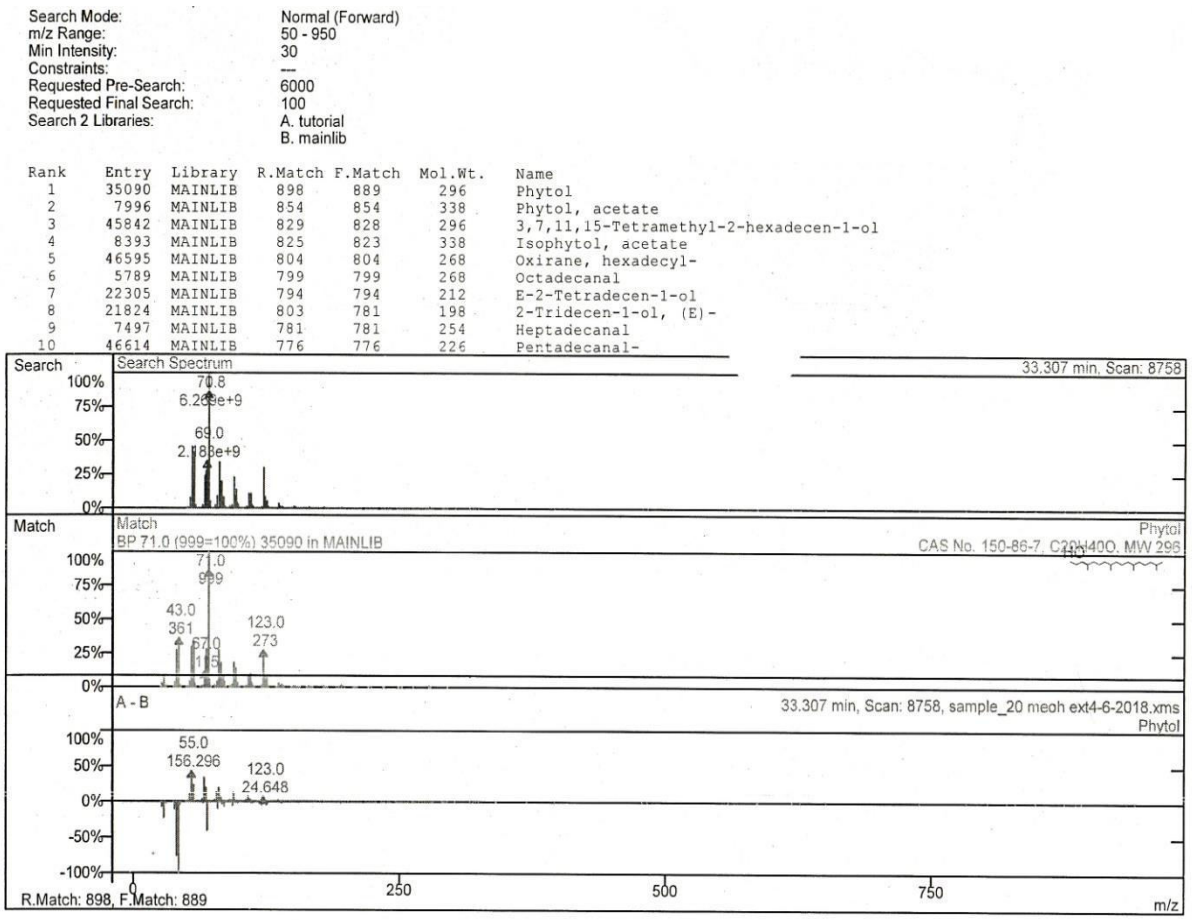
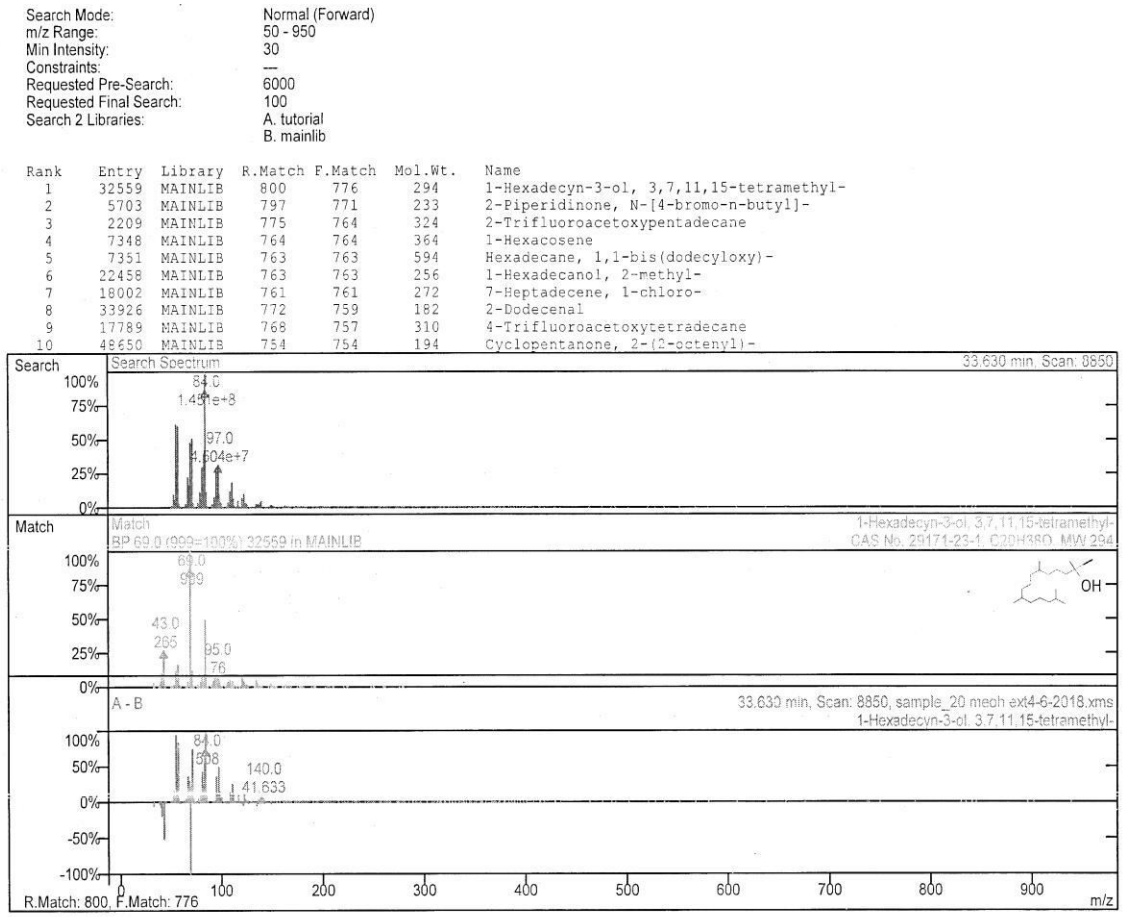


Figure: 21.19 GC-MS profile of proposed lutein

PEAK 18



Search Mode: Normal (Forward)
m/z Range: 50 - 950
Min Intensity: 30
Constraints: ---
Requested Pre-Search: 6000
Requested Final Search: 100
Search 2 Libraries: A. tutorial
B. mainlib

Rank	Entry	Library	R.Match	F.Match	Mol.Wt.	Name
1	32559	MAINLIB	800	776	294	1-Hexadecyn-3-ol, 3,7,11,15-tetramethyl-
2	5703	MAINLIB	797	771	233	2-Piperidinone, N-[4-bromo-n-butyl]-
3	2209	MAINLIB	775	764	324	2-Trifluoroacetoxypentadecane
4	7348	MAINLIB	764	764	364	1-Hexacosene
5	7351	MAINLIB	763	763	594	Hexadecane, 1,1-bis(dodecyloxy)-
6	22458	MAINLIB	763	763	256	1-Hexadecanol, 2-methyl-
7	18002	MAINLIB	761	761	272	7-Heptadecene, 1-chloro-
8	33926	MAINLIB	772	759	182	2-Dodecenal
9	17789	MAINLIB	768	757	310	4-Trifluoroacetoxytetradecane
10	48650	MAINLIB	754	754	194	Cyclopentanone, 2-(2-octenyl)-

Figure: 21.20 GC-MS profile of proposed lutein

PEAK 19

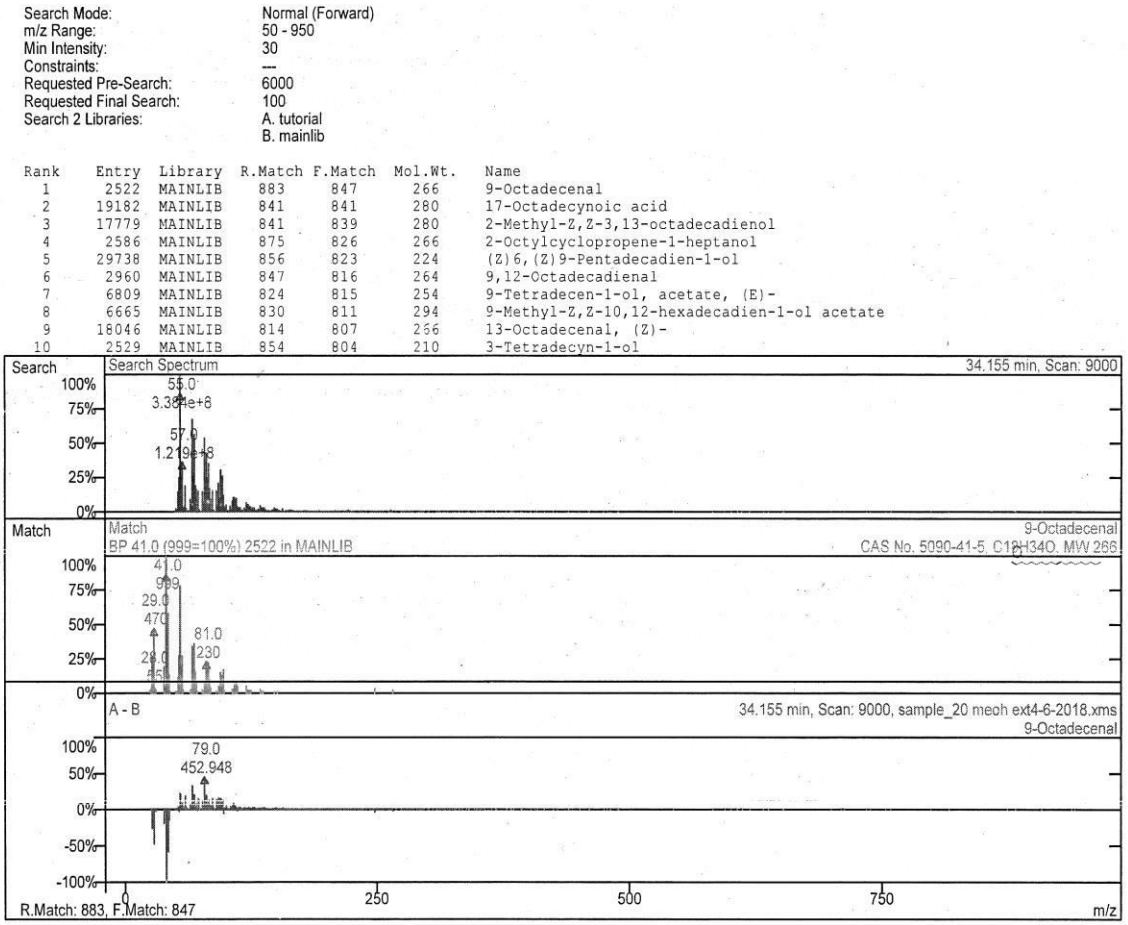


Figure: 21.21 GC-MS profile of proposed lutein

PEAK 20

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Min Intensity: 30
Constraints: ---
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Requested Final Search: 100
Search 2 Libraries: A. tutorial
B. mainlib

Rank	Entry	Library	R.Match	F.Match	Mol.Wt.	Name
1	37108	MAINLIB	756	745	394	Cyclopropanetetradecanoic acid, 2-octyl-, methyl ester
2	17988	MAINLIB	743	743	282	E-11-Hexadecenoic acid, ethyl ester
3	52412	MAINLIB	738	734	368	Docosanoic acid, ethyl ester
4	52472	MAINLIB	727	727	298	Ethyl 14-methyl-hexadecanoate
5	52719	MAINLIB	735	726	340	Eicosanoic acid, ethyl ester
6	52748	MAINLIB	747	725	278	10-Bromodecanoic acid, ethyl ester
7	2255	MAINLIB	720	720	310	Cyclopropanepentanoic acid, 2-undecyl-, methyl ester, trans-
8	52393	MAINLIB	721	716	200	Nonanoic acid, 5-methyl-, ethyl ester
9	17836	MAINLIB	716	716	310	(E)-9-Octadecenoic acid ethyl ester
10	52718	MAINLIB	704	704	228	Dodecanoic acid, ethyl ester

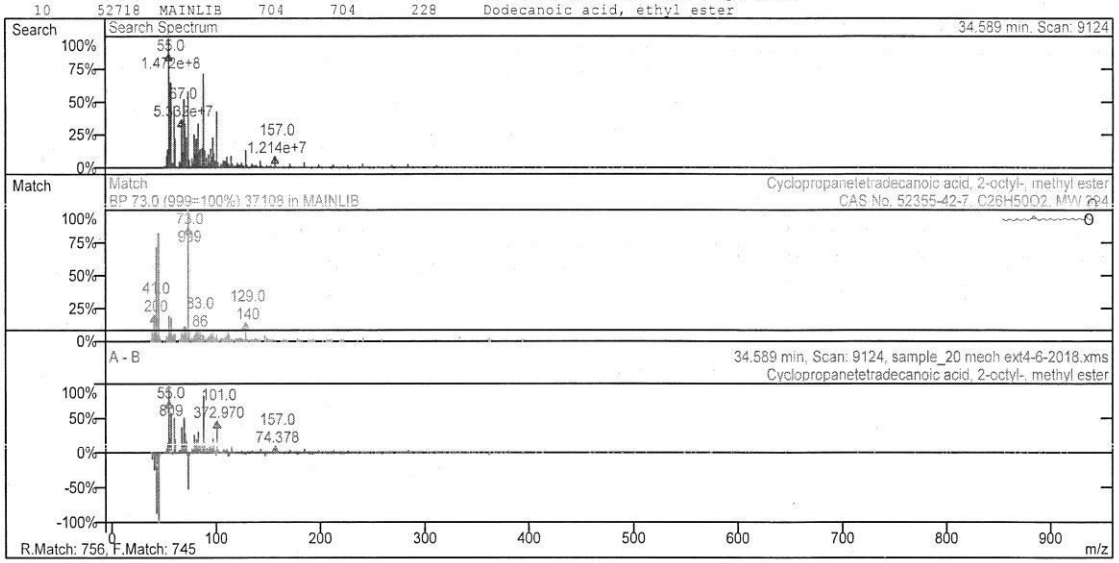


Figure: 21.22 GC-MS profile of proposed lutein

PEAK 21

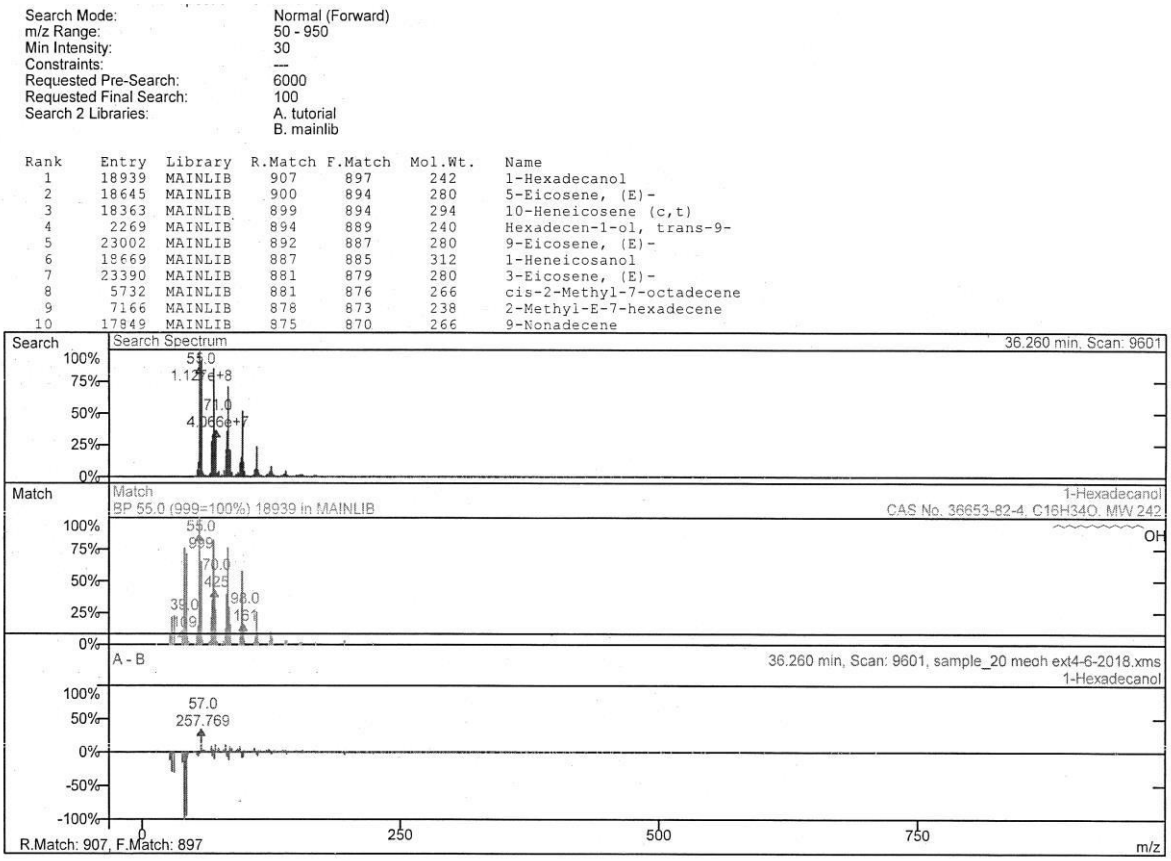


Figure: 21.23 GC-MS profile of proposed lutein

PEAK 22

Search Mode: Normal (Forward)
m/z Range: 50 - 950
Min Intensity: 30
Constraints: ---
Requested Pre-Search: 6000
Requested Final Search: 100
Search 2 Libraries: A. tutorial
B. mainlib

Rank	Entry	Library	R.Match	F.Match	Mol.Wt.	Name
1	22563	MAINLIB	927	927	414	Heptacosane, 1-chloro-
2	23579	MAINLIB	912	912	604	Tritetracontane
3	9288	MAINLIB	906	906	376	Sulfurous acid, hexyl pentadecyl ester
4	23666	MAINLIB	904	904	320	Sulfurous acid, butyl tridecyl ester
5	35062	MAINLIB	903	903	334	Sulfurous acid, pentyl tridecyl ester
6	23475	MAINLIB	900	900	306	Sulfurous acid, butyl dodecyl ester
7	35063	MAINLIB	896	896	348	Sulfurous acid, pentyl tetradecyl ester
8	35064	MAINLIB	895	895	362	Sulfurous acid, pentadecyl pentyl ester
9	35065	MAINLIB	894	894	376	Sulfurous acid, hexadecyl pentyl ester
10	15971	MAINLIB	892	892	272	Methoxyacetic acid, 3-tridecyl ester

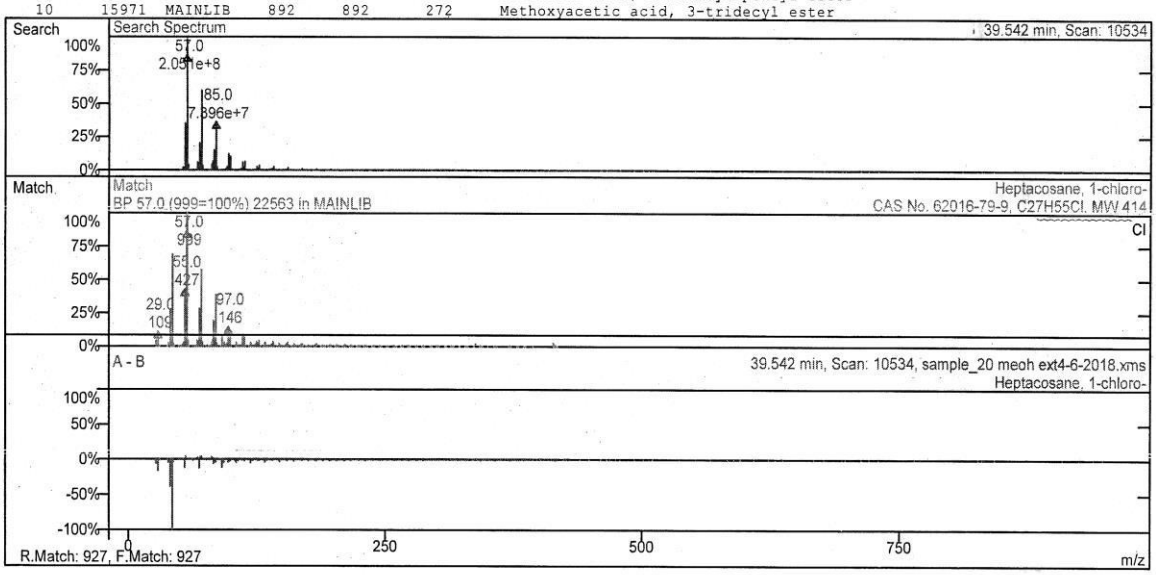
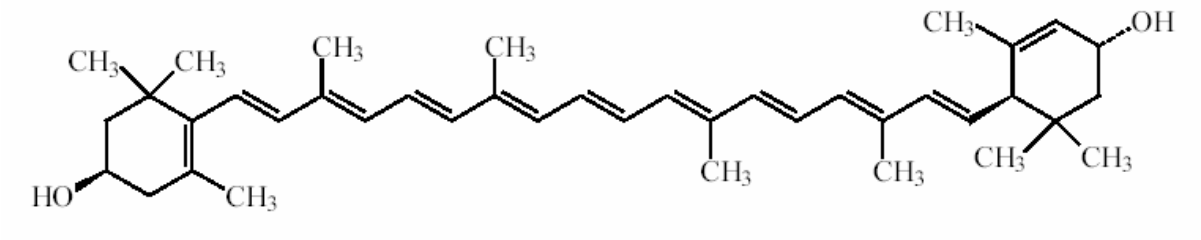


Figure: 21.24 GC-MS profile of proposed lutein

STRUCTURE OF LUTEIN



IUPAC NAME:β,ε-carotene-3,3'-diol

Other name

4-[18-(4-Hydroxy-2,6,6-trimethyl-1-cyclohexenyl)-3,7,12,16-tetramethyloctadeca 1,3,5,7,9,11,13,15,17-nonaenyl]-3,5,5-trimethyl-cyclohex-2-en-1-olXanthophyll

Chemical formula : $C_{40}H_{56}O_2$

Appearance : Red – orange crystalline solid

Solubility in water : Insoluble

Solubility in fats : Soluble

Table: 18 GC-MS ANALYSIS OF PROPOSED LUTEIN

Sl.No	Peak No	Retention Time (minutes)	Molecular Weight	Name of the compound
1	1	8.654	124	3**-Octyne, 7-methyl-**
2	2	9.091	128	Naphthalene
3	3	11.645	212	Pentadecane
4	4	14.919	184	Tridecane
5	5	18.275	220	ButylatedHydroxy Toluene
6	6	21.683	212	**Pentadecane**
7	7	23.775	262	Sulfurous acid, cyclohexylmethyl hexyl ester
8	8	24.212	296	Heptadecane, 2,6,10,15-tetramethyl-
9	9	25.388	272	Methoxyacetic acid, 3-tridecyl ester
10	10	26.731	296	1-Iodo-2-methylundecane
11	11	27.717	268	2-Pentadecanone, 6,10,14-trimethyl-
12	12	29.507	284	**Hexadecanoic acid, 15-methyl-, methyl ester**
13	13	30.106	278	Dibutyl phthalate
14	14	30.872	340	Eicosanoic acid, ethyl ester
15	15	31.153	256	n-Hexadecanoic acid

16	16	32.672	326	**Hexadecanoic acid, 3,7,11,15-tetramethyl-, methyl ester**
17	17	33.307	296	Phytol
18	18	33.630	294	**1-Hexadecyn-3-ol, 3,7,11,15-tetramethyl-**
19	19	34.155	266	**9-Octadecenal**
20	20	34.589	394	**Cyclopropanetetradecanoic acid, 2-octyl-, methyl ester**
21	21	36.260	242	1-Hexadecanol
22	22	39.542	414	Heptacosane, 1-chloro-

Some of the peaks were relevant with molecular weight in the GC-MS profile(**Figure: 21-21.24**), identified the presence of certain linkages of lutein.Hence the derived Ultraviolet spectrum(**Table 13&14**), Nuclear Magnetic Resonance (^{1}H NMR, ^{13}C NMR) spectrum(**Table15,16&17**) and GC-MS profile(**Table:18**) confirmed the molecular weight of lutein and also supports the additional research work indicating the chemical structure of lutein from the leaf powder. (**Omayma A. Eldahshan et al., 2013 &Finar IL, 1975**)

DETERMINATION OF SUN PROTECTION FACTOR OF ISOLATED LUTEIN FROM *Commelinabenghalensis L.*

Isolated compound lutein was subjected to sun protection factor determination and the results are showed in **Table: 20, 21 & 22.**

SUN PROTECTION FACTOR

Table: 19 Values of EE * I used in the calculation of SPF

Wavelength (λ nm)	EE * I (Normalized)
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180
Total	**1**

EE – Erythemal effect spectrum , I – solar intensity spectrum

SPF can be calculated by applying the following formula known as Mansur equation (Kaur and Saraf, 2010; Mishra *et al.,* 2012):

$$\mathbf{SPF = CF \times \sum_{290}^{320} EE\ (\lambda)\ \times\ I\ (\lambda)\ \times\ Abs\ (\lambda)}$$

Where CF = correction factor (10), EE (λ) = erythmogenic**e**ffect of radiation with wavelength λ, Abs (λ) =Spectrophotometric absorbance values at wavelength λ. The values of EE x λ are constants.

DETERMINATION OF SUN PROTECTION FACTOR OF ISOLATED LUTEIN FROM *Commelinabenghalensis L.* (LUTEIN 50,100,200 µg/ml)

Table: 20Lutein 50 µg/ml

Sl.no	Wavelength (λ nm)	EE * I (Normalized)	Absorbance (Lutein 50µg/ml) Mean ± SEM	SPF value
1	290	0.0150	0.119 ± 0.0017	0.01785
2	295	0.0817	0.115 ± 0.0003	0.093955
3	300	0.2874	0.116 ± 0.0003	0.333384
4	305	0.3278	0.113 ± 0.0003	0.370414
5	310	0.1864	0.114 ±0.0006	0.212496
6	315	0.0839	0.119 ± 0.0003	0.099841
7	320	0.0180	0.122 ± 0.0003	0.02196
			SPF	1.1499 ± 0.43

Table: 21Lutein 100 µg/ml

Sl.no	Wavelength (λ nm)	EE * I (Normalized)	Absorbance Lutein (100 µg/ml) Mean ± SEM	SPF value
1	290	0.0150	0.241 ± 0.0003	0.03615
2	295	0.0817	0.241 ± 0.0003	0.196897
3	300	0.2874	0.238 ± 0.0003	0.684012
4	305	0.3278	0.234 ± 0.0003	0.767052
5	310	0.1864	0.234 ± 0.0003	0.436176
6	315	0.0839	0.252 ± 0.0006	0.211428
7	320	0.0180	0.262 ± 0.0006	0.04716
			SPF	2.378875 ± 0.90

Table: 22Lutein 200 µg/ml

Sl.no	Wavelength (nm)	EE * I (Normalized)	Absorbance Lutein (200 µg/ml) Mean ± SEM	SPF value
1	290	0.0150	0.495 ± 0.0006	0.07425
2	295	0.0817	0.484 ± 0.0003	0.395428
3	300	0.2874	0.475 ± 0.0006	1.36515
4	305	0.3278	0.463 ± 0.0003	1.517714
5	310	0.1864	0.475 ± 0.0003	0.8854
6	315	0.0839	0.495 ±0.0006	0.415305
7	320	0.0180	0.511 ±0.0006	0.09198
			SPF	4.745227 ± 1.79

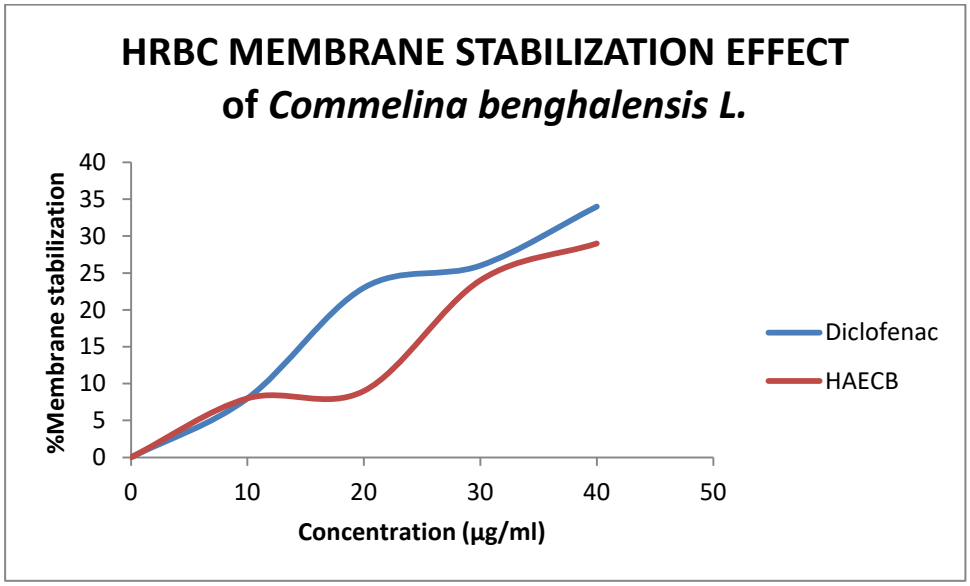
The SPF number of lutein 50µg/ml is 1.15 ± 0.43. The SPF number of lutein 100µg/ml is 2.38 ± 0.90. The SPF number of lutein 200µg/ml is 4.75 ± 1.79. .

SPF value for sunscreen above 2 isconsidered as having good sunscreen activity.Marketed sunscreen lotion having concentration 200µg/ml shows SPF value about 0.66±0.006. (Manoj A. Suva., 2014)

ANTI-INFLAMMATORY ACTIVITY

Hydroalcoholic extract of *Commelinabenghalensis L.* was subjected to Anti-Inflammatory activity by Membrane stabilization method.

FIGURE:22 PERCENTAGE OF MEMBRANE STABILIZATION BY DICLOFENAC SODIUM AND HAECB



The inhibitory concentration (IC_{50}) of *Commelinabenghalensis L.* (Leaf) in HRBC membrane stabilization study is found to be 69µg/mLin comparison with diclofenac sodium57µg/mL.

Table:23 PERCENTAGE OF MEMBRANE STABILIZATION BY DICLOFENAC SODIUM AND HAECB

S.NO	Concentration (µg/ml)	Percentage membrane stabilization of Diclofenac*	Percentage membrane stabilization of HAECB*
1	10	8.14 ± 0.0088	7.7 ± 0.0577
2	20	22.75 ± 0.0088	9 ± 0.0577
3	30	25.9 ± 0.0088	23.5 ± 0.1453
4	40	34.4 ± 0.3333	29.3 ± 0.0882
	IC_{50}	57 µg/mL	69 µg/mL

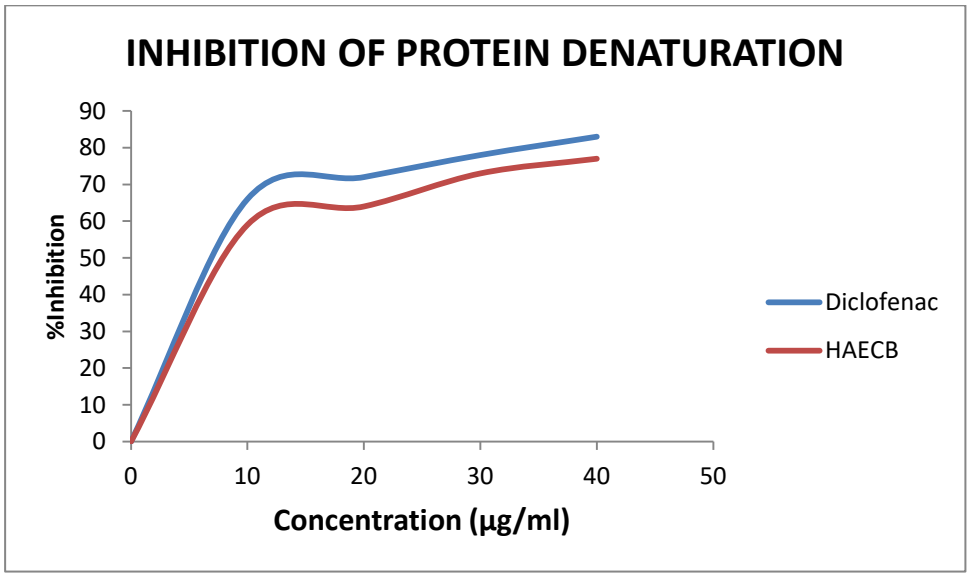
*Mean of three readings ± SEM

HAECB showed mild anti-inflammatory activity.

ANTI-ARTHRITIC ACTIVITY

Hydroalcoholic extract of *Commelinabenghalensis L.* was subjected to Anti-Arthritic activity by Protein denaturation method.

Figure: 23Effect of HAECB and Diclofenac Sodium on Inhibition of Protein Denaturation



The inhibitory concentration (IC_{50}) of *Commelinabenghalensis L.* (Leaf) in Protein denaturation is found to be 17µg/mL in comparison with diclofenac sodium 14µg/mL.

Table: 24 Effect Of HAECB and Diclofenac Sodium on Inhibition of Protein Denaturation

Sl. NO	Concentration in µg/ml	% Inhibition	
		Diclofenac	HAECB
1	10	65.6 ± 0.3464	59.4 ± 0.2309
2	20	71.9 ± 0.5774	64.1 ± 0.0882
3	30	78.1 ± 0.0882	73.4 ± 0.2309
4	40	82.8 ± 0.5774	76.6 ± 0.1732
	IC_{50}	14 µg/mL	17 µg/mL

*Mean of three readings ± SEM

HAECB showed moderate anti-arthritic activity.

INVITRO ANTIOXIDANT STUDIES

Hydroalcoholic extract was subjected to in-vitro antioxidant studies. It includes hydrogen peroxide scavenging activity, Reducing power assay, total antioxidant capacity.

Determination of Hydrogen peroxide scavenging activity of *Commelinabenghalensis L.* (leaf) (HAECB)

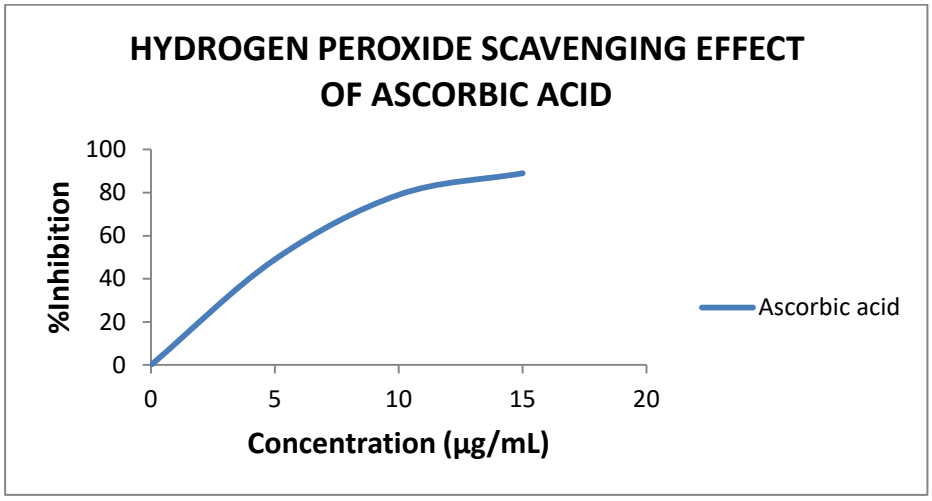


Figure: 24 Hydrogen Peroxide Scavenging Effect of Ascorbic Acid

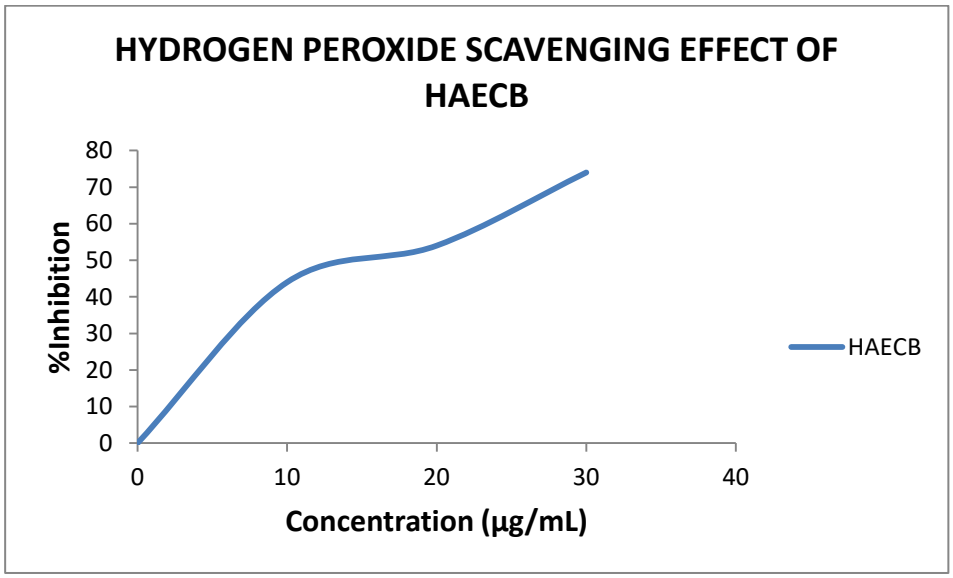


Figure: 25 Hydrogen Peroxide Scavenging Effect of HAECB

The inhibitory concentration (IC_{50}) of *Commelinabenghalensis L.*(Leaf) against hydrogen peroxide scavenging effect is found to be 18 µg/mL in comparison with ascorbic acid 7µg/mL.

Table: 25 Determination of Hydrogen peroxide scavenging activity of *Commelinabenghalensis L.* (leaf) (HAECB)

S.no	Concentration		Percentage inhibition of Ascorbic acid	Percentage inhibition of *Commelinabenghalensis L.* (µg/mL)
	Ascorbic acid (µg/mL)	*Commelina benghalensis L.* (µg/mL)		
1	5	10	49 ± 0.333	44 ± 0.333
2	10	20	79 ± 0	54 ± 0
3	15	30	89 ± 0	74 ± 0
	IC_{50}		7 µg/mL	18 µg/mL

It requires the double the concentration of ascorbic acid to reduce the free radicals. It showed mild anti oxidant effect when compared with ascorbic acid. It requires the double the concentration of ascorbic acid to scavenge the free radicals generated.

Determination of Reducing power assay of *Commelinabenghalensis L.* (leaf) (HAECB)

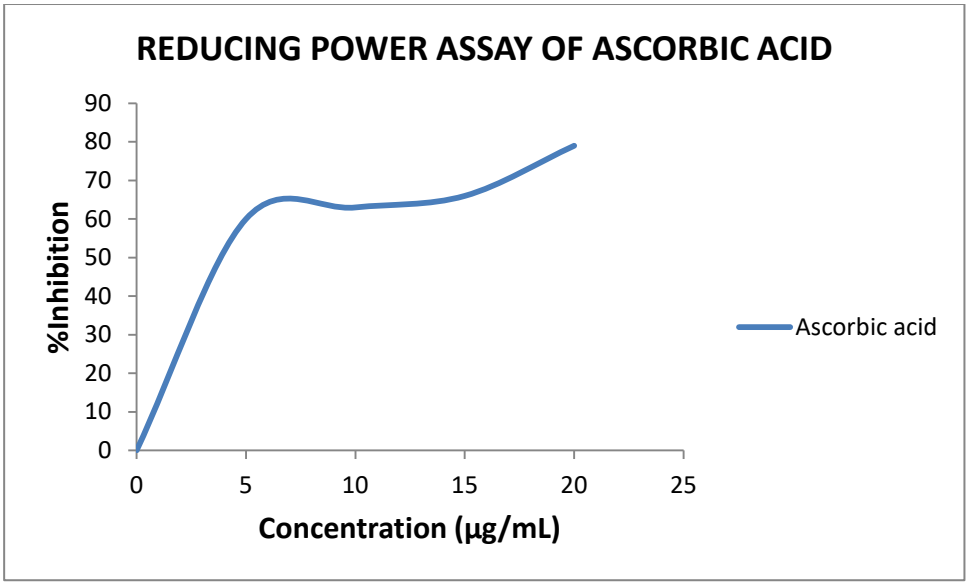


Figure: 26 Reducing Power Assay of Ascorbic Acid

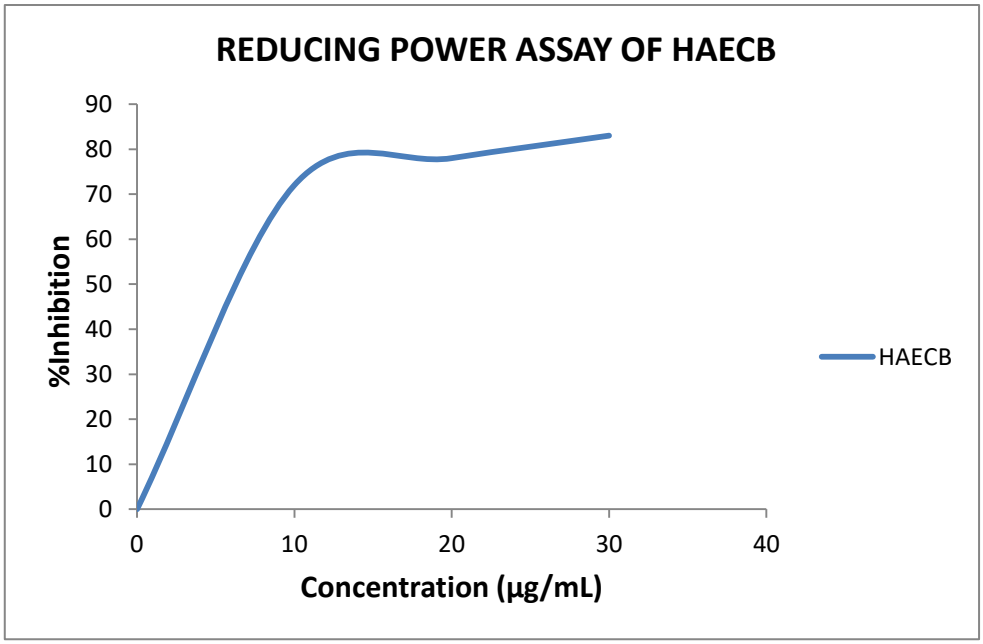


Figure: 27 Reducing Power Assay of HAECB

The inhibitory concentration (IC_{50}) of *Commelinabenghalensis L.*(Leaf) against **reducing power assay** determinedin comparison with ascorbic acid used as a standard. The inhibitory concentration (IC_{50}) of *Commelina benghalensis L.*(Leaf) in reducing power assay is found to be 12 µg/mL in comparison with ascorbic acid 9µg/mL.

TABLE: 26Determination of Reducing Power Assay of *CommelinaBenghalensis L.* (Leaf) (HAECB)

S.no	**Concentration**		**Percentage inhibition of Ascorbic acid**	**Percentage inhibition of *Commelinabenghalensis L.* (µg/mL)**
	Ascorbic acid (µg/mL)	***Commelina benghalensis L.* (µg/mL)**		
1	5	10	60 ± 0.333	72 ± 0.333
2	10	20	63 ± 0.333	78 ± 0.333
3	1530		66 ± 0.333	83 ± 0.333
	20		79 ± 0.333	
	IC_{50}		9 µg/mL	12 µg/mL

It showed moderate antioxidant effect when compared with ascorbic acid.

Determination of Total antioxidant capacity of *Commelinabenghalensis L.* (leaf) (HAECB)

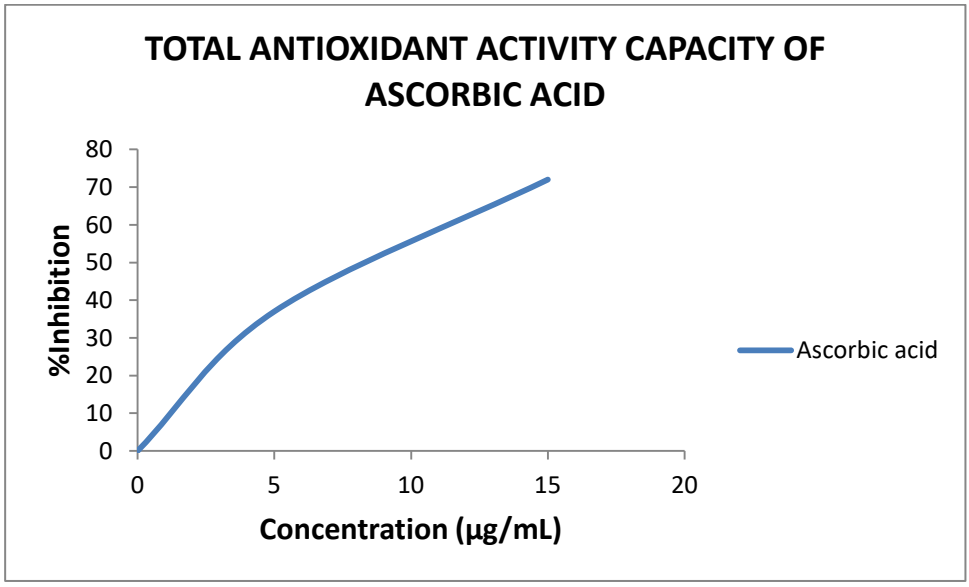


FIGURE: 28 TOTAL ANTIOXIDANT ACTIVITY OF ASCORBIC ACID

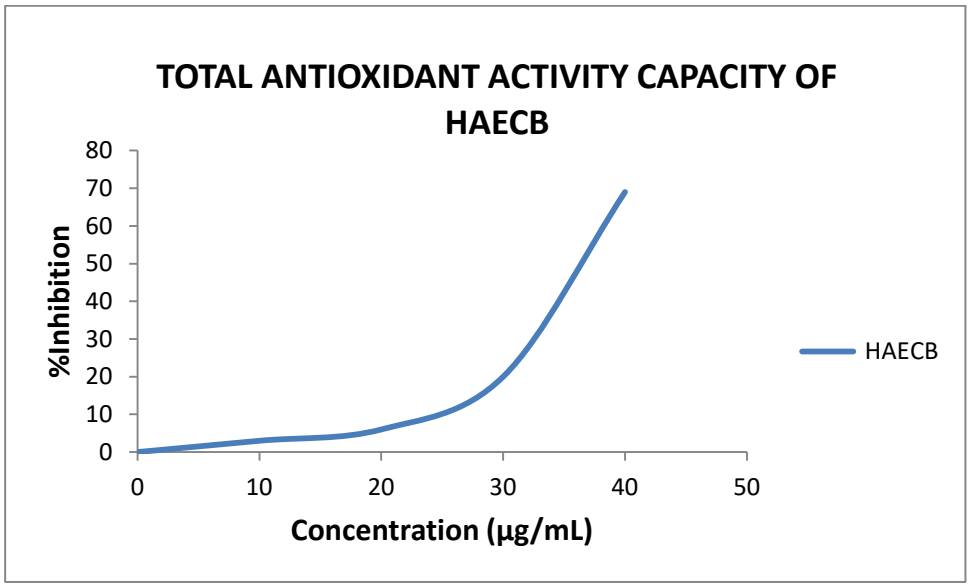


FIGURE: 29 TOTAL ANTIOXIDANT ACTIVITY OF HAECB

The inhibitory concentration (IC_{50}) of *Commelinabenghalensis L.*(Leaf) against **total antioxidant capacity** was determined in comparison with ascorbic acid used as a standard. The total antioxidant capacity is found to be 40 µg/mL in comparison with ascorbic acid 10 µg/mL.

Table: 27 Determination of Total Antioxidant Capacity of *Commelinabenghalensis L.* (Leaf) (HAECB)

S.no	**Concentration**		**Percentage inhibition of Ascorbic acid**	**Percentage inhibition of *Commelinabenghalensis L.* (µg/ml)**
	Ascorbic acid (µg/ml)	***Commelina benghalensis L.* (µg/ml)**		
1	5	10	37 ± 0.882	3 ± 0.577
2	15	20	72 ± 0.577	6 ± 0.577
3	30			20 ± 0.667
	40			69 ± 0.577
	IC_{50}		10 µg/mL	40 µg/mL

Concentration of hydroalcoholic extract of *Commelinabenghalensis L.* requires 4 times the amount of ascorbic acid to reduce the free radicals. It showed mild anti oxidant effect when compared with ascorbic acid. It requires the 4 times the concentration of ascorbic acid to scavenge the free radicals generated.

CHAPTER-6

SUMMARY

SUMMARY

Chapter 1

It consists of introduction of the present thesis. It includes the importance of plant and diagnosis, cure & prevent the disease.

Chapter 2

It consists of literature review of the present thesis. It includes ethno claim review, phytochemical review, pharmacological review. Ethno claim review such as plant part used for various disease. Phytochemical review is used for the presence of active constituents detected previously. Pharmacological review helps to identify the activity studied so far.

Chapter 3

It contains aims and objective of the present thesis. The aim of present research is to study the Pharmacognostical, Phytochemical studies includes isolation of lutein and Pharmacological evaluation of sun protection factor of isolated lutein, and anti-inflammatory, anti-arthritic activity of *Commelina benghalensis L.*

Chapter 4

It consists materials and methods of the present thesis. It is divided into part A,B,C.

Part A includes pharmacognostical studies, macroscopy of *Commelina benghalensis L.* (Leaf), quantitative microscopy, determination of physio - chemical constants, behavioural characters of the *Commelina benghalensis L.* (leaf- crude powder) with different reagents, determination of physical parameters of (HAECB),

Part B includes phytochemical studies, preliminary phytochemical screening of hydro-alcoholic extract of *Commelina benghalensis L.* (leaf), TLC profile, determination of tannic acid equivalent in (HAECB), determination of gallic acid content equivalent in (HAECB), determination of rutin equivalent in (HAECB), determination of chlorophyll "a", chlorophyll "b", total chlorophyll and total carotenoid, UV report and NMR spectrum report of carotenoids (lutein).

Part C includes pharmacological studies. invitro anti-inflammatory studies, antiarthritic studies , sun protection factor studies of isolated lutein, and anti-oxidant study.

Chapter 5

It consist results and discussion of the present research thesis. It includes the result for macroscopy of *Commelina benghalensis L.*(Leaf), quantitative microscopy, Stomatal Number - (lower epidermis - minimum 39, maximum 48, average 42.6) (upper epidermis - minimum 15, maximum 20, average 17.8). Stomatal Index- (lower epidermis - minimum 30.7, maximum 34.5, average 32.0) (upper epidermis - minimum 13.8, maximum 20.8, average 17.32).

Determination of physio – chemical constants, The Physio – chemical constants of *Commelina benghalensis L.* (Leaf – crude powder) was found to be, Loss on drying (0.78 ± 0.11% w/w), Total Solids (99.22 ± 0.11 % w/w). The powder did not possess any foreign matter, bitter principle and volatile oil content. The powder also exhibits Petroleum ether extractive value (0.6 ± 0.12% w/w), Ethyl acetate extractive values (1.6 ± 0.12% w/w), Chloroform extractive value (1.7 ± 0.07% w/w), Methanol extractive values (4.4 ± 1.29% w/w), Aqueous extractive values (12.13 ± 1.30% w/w), Total ash value (16 % w/w), Water soluble ash value (10.5 % w/w), Acid insoluble ash value (1.17 % w/w)

Behavioural characters of the *Commelina benghalensis L*. (Leaf – crude powder) with different reagents, determination of physical parameters of (HAECB), Crude powder when treated with Water, Con.HCl, Con H_2SO_4, CH_3COOH, Con HCl + Water, Con H_2SO_4 + Water and Aqueous NaOH, Aqueous $Fecl_3$ showed **black colour** in visible light. Powder treated with Con.HNO_3 and Con.HNO_3 + Water showed **yellow colour** in visible light. Powder treated with CH_3COOH + Water showed yellowish **brown colour** in visible light. Powder when treated with Water and Con.HCl showed **brown colour** in UV 254 nm. Powder treated with Con.H_2SO_4 showed **dull brown** colour in UV 254 nm. Powder treated with Con.HNO_3 showed **green colour** in UV 254 nm. Powder when treated with CH_3COOOH, Con.HCl + Water, Con.H_2SO_4 + Water, Con.HNO_3 + Water, CH_3COOH + Water, Aqueous NaOH, Aqueous $Fecl_3$ showed **dull green** colour in UV 254 nm. The powder did not show any characteristic colour change with the reagent under UV 366 nm.

Determination of physical parameters of (HAECB), The physical parameters of hydro alcoholic extracts of *Commelina benghalensis L.* (Leaf) such as refractive index, weight per ml, consistency and colour was determined. It was found to be refractive index (1.342 ± 0.002), weight per ml (0.882 ± 0.010), dark brown in colour with semisolid consistency.

The phytochemical screening of the hydroalcoholic extract (70%) of *Commelina benghalensis L.* (Leaf) powder revealed the presence of alkaloids, carbohydrates, sterols, saponins, tannins and phenolic compound, flavonoids, protein and free aminoacid, terpenoids, mucilage, betacyanin, quinone, phlobatannins, carotenoids. It shows the absence of anthraquinone glycosides, cardiac glycoside, fixed oil, anthocyanin, lecoanthocyanin, emodin, gum, resins, volatile oil. The aqueous extract of *Commelina benghalensis L.* (Leaf) powder revealed the presence of alkaloids, carbohydrates, sterols, saponins, tannins and phenolic compound, flavonoids, protein and free aminoacid, mucilage, emodin, quinone, phlobatannins,

carotenoids. It shows the absence of anthraquinone glycosides, cardiac glycoside, terpenoids, fixed oil, betacyanin, gum, anthocyanin, lecoanthocyanin, resins, volatile oil.

TLC profile

Thinlayer chromatography of the hydroalcoholic extract of *Commelina benghalensis L.* (Leaf) showed the R_f value 0.25(Gallic acid standard) 0.25, 0.90 (HAECB) may indicate the presence of phenolic compounds the solvent system used in **Acetic acid : Chloroform (1:9)**. R_f value 0.72 (Gallic acid standard), 0.73, 0.90 (HAECB) may indicate the presence of phenolic compounds the solvent system used in **Ethyl acetate : Benzene (9:11)**. R_f value 0.46 (Rutin standard) 0.71 (HAECB), may indicate the presence of flavonoids the solvent system used in **Chloroform : Ethyl Acetate (60:40)**. R_f value 0.42 (Rutin standard), 0.45,0.93,0.97 (HAECB) may indicate the presence of flavonoids, flavones, flavonols the solvent system used in **Ethyl Acetate : Formic Acid : Glacial Acetic Acid : Water (100:11:11:26)**.

Determination of gallic acid content equivalent in (HAECB), in terms of (GAE) was found to be 112 mg/g. Determination of tannic acid equivalent in (HAECB), in terms of (TAE) was found to be 198 mg/g. Determination of rutin equivalent in (HAECB), in terms of (RE) was found to be 989mg/g.

Determination of Chlorophyll “a”, Chlorophyll “b”, Total chlorophyll and Total carotenoid content in fresh leaves of *Commelina benghalensis L.* It was found to be Chlorophyll “a” (0.00311 mg/gm), Chlorophyll “b” (0.017506 mg/gm), Total chlorophyll (0.052588 mg/gm) and Total carotenoid (0.06878 mg/gm).

UV report of carotenoids (lutein)

Lutein and Chlorophyll, Aliquot of crude extract was transferred to cuvette and was scanned under UV range from 300 – 700 nm in the UV – Visible spectrophotometer to check the

presence of chlorophyll and lutein. It shows the absorbance maxima at 665, 466, 412 nm. It indicates the presence of chlorophyll (665 nm) and lutein (466, 412 nm)

Lutein, the isolated compound was dissolved in diethyl ether and transferred to cuvette and was scanned under UV range from 300 – 700 nm in the UV – Visible spectrophotometer. The isolated compound lutein showed absorbance maxima at 470 and 444 nm indicates the presence of lutein.

NMR studies

The Isolated compound was dissolved in Duterated chloroform ($CDCL_3$) and it showed the chemical shift value of ^1H NMR

^1H NMR: (300 MHz, $CDCl_3$) δ_H 0.78(s, 10H), 0.94 - 1.00 (bd, 2H), 1.12 - 1.35 (bm, 21H), 1.53-1.60 (bd, 3H), 1.81-1.90 (bd, 8H), 3.38-3.45 (m, 2H), 4.73 (s, 1H).

The Isolated compound was dissolved in Duterated chloroform ($CDCL_3$) and it showed the chemical shift value for ^{13}C NMR.

^{13}C NMR: (75 MHz, $CDCl_3$); δ_C 15.3, 19.7, 19.9, 22.6, 22.7, 24.8, 25.2, 28.0, 29.7, 31.9, 32.7, 32.8, 36.7, 37.3, 37.5, 38.4, 65.9, 130.3.

GC-MS studies

Some of the peaks were relevant with molecular weight in the GC-MS profile (Figure: 21-21.24), identified the presence of certain linkages of lutein. Hence the derived Ultraviolet spectrum (Table 13&14), Nuclear Magnatic Resonance (^1H NMR, ^{13}C NMR) spectrum (Table15,16&17) and GC-MS profile (Table:18) confirmed the molecular weight of lutein and also supports the additional research work indicating the chemical structure of lutein from the leaf powder.

Determination of Sun protection factor

The SPF number of lutein 50µg/ml is found to be 1.15 ± 0.43. The SPF number of lutein 100µg/ml is found to be 2.38 ± 0.90. The SPF number of lutein 200µg/ml is found to be 4.75 ± 1.79. .

SPF value for sunscreen above 2 is considered as having good sunscreen activity. Marketed sunscreen lotion having concentration 200 µg/ml shows SPF value about 0.66±0.006. (Manoj A. Suva., 2014)

Invitro membrane stabilization study

Lysosomes are intracellular particles which contain most of the lytic and digestive enzymes of the tissue. The rupture of the lysosomes results in injury or death to surrounding tissues and also acute inflammation. The membranes of lysosomes and erythrocytes are destroyed by similar agents; hence a test was developed to measure the ability of compounds to stabilize erythrocyte membrane to heat hemolysis. RBC when exposed to various injurious substances such as methyl salicylate, phenyl hydrazine, and hypotonic medium or over heat will cause lysis of membrane accompanied by haemolysis and oxidation of haemoglobin. RBC membranes are easily susceptible to free radical mediated lipid peroxidation by breakdown of biomolecules. Due to it has rich source of iron and high oxygen partial pressure. RBC membranes are similar to lysosomal cells. All NSAIDs inhibited hemolysis while other type of compounds had no effect. Hence prevention of hypotonic and heat mediated RBC membrane lysis taken as measure of anti-inflammatory activity of drugs.

A study has reported that the flavonoids exert membrane stabilizing effect on lysosomes both invitro and invivo in experimental animals. Another report has suggested that tannins and saponins have the ability to bind cations and other biomolecules and are able to stabilize the erythrocyte membrane. HAECB extract is highly potent on human erythrocyte and thus adequately protecting it against heat and hypotonicity induced lysis. The inhibitory concentration (IC_{50}) of *Commelina benghalensis L.* (Leaf) in HRBC membrane stabilization study is found to be 69µg/ml in comparison with diclofenac sodium 57µg/ml. It showed mild anti-inflammatory activity.

The phytochemical analysis showed that the HRBC has flavonoids and tannins. Hence the HRBC membrane stabilizing capacity may be due to the presence of the above mentioned constituents which will prevent the oxidation of haemoglobin and also due to its antioxidant property.

Invitro antiarthritic activity by protein denaturation method

The principle involved is the inhibition of protein denaturation. Denaturation of protein was found to be one of the causes of rheumatoid arthritis. In rheumatoid arthritis, the production of autoantigen may be due to protein denaturation which involves the alteration of electrostatic hydrogen, hydrophobic and disulphide bonding.

The protein used in this study is bovine serum albumin. Denaturation of protein is carried out by heating. The aim of this activity is to inhibit denaturation and to exhibit protective effect against rheumatoid arthritis. The inhibitory concentration (IC_{50}) of *Commelina benghalensis L.* (Leaf) in Protein denaturation is found to be 17µg/ml in comparison with diclofenac sodium 14µg/ml. It showed moderate anti-arthritic activity. The inhibition of protein denaturation by HAECB may be due to the presence of phenolic compounds, flavonoids and tannins.

Anti-oxidant study

It includes four methods. Determination of scavenging activity against hydrogen peroxide, Reducing power assay, determination of total antioxidant activity. The inhibitory concentration (IC_{50}) of *Commelina benghalensis L*. (leaf) against hydrogen peroxide scavenging effect is found to be 18µg/ml in comparison with ascorbic acid 7µg/ml. The inhibitory concentration (IC_{50}) of *Commelina benghalensis L*. (leaf) against reducing power assay determined in comparison with ascorbic acid used as a standard. The inhibitory concentration (IC_{50}) of *Commelina benghalensis L*. (leaf) in reducing power assay is 12µg/mL in comparison with ascorbic acid 9µg/mL. The inhibitory concentration (IC_{50}) of *Commelina benghalensis L*. (leaf) against total antioxidant capacity was determined in comparison with ascorbic acid used as a standard. The total antioxidant capacity is found to be 40µg/ml in comparison with ascorbic acid 10µg/ml.

Chapter 6 It consists of summary of the present thesis.

Chapter 7 It includes the conclusion.

CHAPTER-7

CONCLUSION

CONCLUSION

- The present investigation draws the following conclusion (succeeding) inference.
- Pharmacognostical parameters for the leaves of *Commelina benghalensis L*. Hexacytic stomato and raphides are the diagnostic features of *Commelina benghalensis L*.
- The derived parameters would help to fix or draw the monographical standards.
- Quantitative estimation helps to identify the gallic acid, tannic acid, and flavonoids equivalents present in the hydroalcoholic extract of *Commelina benghalensis L*.
- Chlorophyll "a", Chlorophyll "b", Total chlorophyll and Total carotenoid content in fresh leaves of *Commelina benghalensis L*. were determined.
- TLC studies recorded the presence of flavonoids, flavones, flavonols and phenolic compounds.
- The phytochemical screening of the hydroalcoholic extract (70%) of *Commelina benghalensis L.* (Leaf) powder revealed the presence of alkaloids, carbohydrates, sterols, saponins, tannins and phenolic compound, flavonoids, protein and free aminoacid, terpenoids, mucilage, betacyanin, quinone, phlobatannins, carotenoids. The aqueous extract of *Commelina benghalensis L.* (Leaf) powder revealed the presence of alkaloids, carbohydrates, sterols, saponins, tannins and phenolic compound, flavonoids, protein and free aminoacid, mucilage, emodin, quinone, phlobatannins, carotenoids.
- Isolation of carotenoids such as Lutein was confirmed by UV, NMR (^{1}H NMR,^{13}C NMR), and GC-MS spectrum.
- Pharmacological screening potentiates the biological invitro anti-inflammatory effect, invitro anti-arthritic effect, invitro sun protection effect of isolated lutein, and invitro-antioxidant effect.
- At the site of inflammation, HAECB may possibly inhibit the release of lysosomal content of neutrophils (bactericidal enzymes and proteinases) which upon extracellular

release cause further tissue inflammation and damage (Chou, 1997). In the present study, results indicate that the HAECB possesses significant anti-inflammatory properties which may be due to the strong occurrence of polyphenolic compounds such as flavonoids, tannins and phenols.

- HAECB showed significant antiarthritic activity by inhibition of protein denaturation. Rheumatoid arthritis (RA) being a common inflammatory disease affects about 1% of the adult population worldwide. It occurs in immunogenetically predisposed individuals. Protein denaturation was found to be one of the causes of RA. HAECB has shown significant anti-arthritic activity and the phenolic constituent may be responsible for this activity.
- Further investigation may be carried out for the bio-active fraction for its potential pharmacological effects.
- Future research studies may be extended to isolate other phyto-constituents present in this plant.

CHAPTER-8

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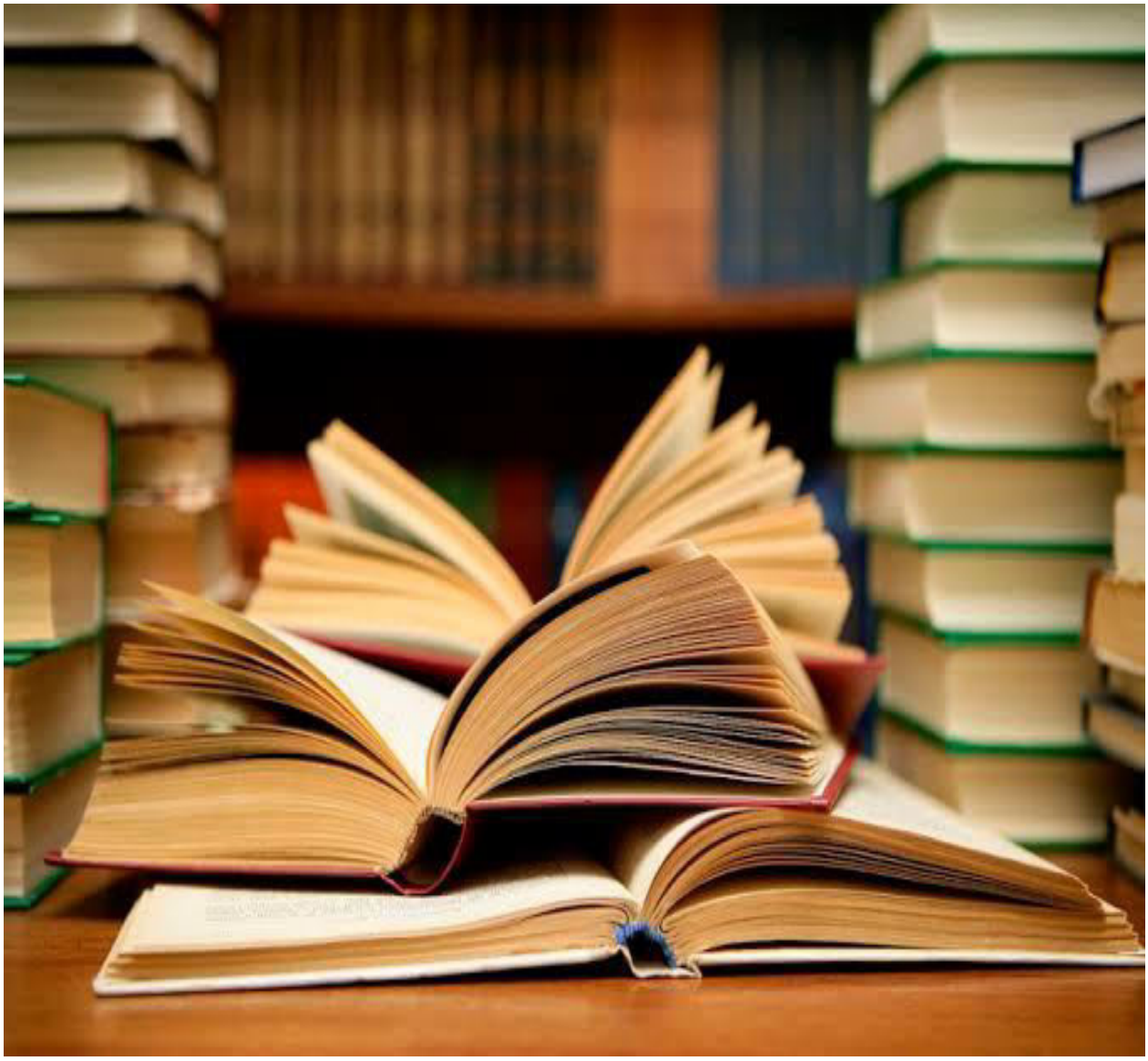

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